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Water Quality Criteria for Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

FINAL REPORT

Elizabeth L. Etnier

JUNE 1986

SUPPORTED BY

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, MD 21701-5012
Interagency Agreement No. 84PP4845

Oak Ridge National Laboratory
Oak Ridge, TN 37831

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Fort Detrick, Frederick, MD 21701-5010

OPERATED BY
MARTIN MARIETTA ENERGY SYSTEMS, INC.
FOR THE UNITED STATES
DEPARTMENT OF ENERGY

Printed in the United States of America. Available from
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road, Springfield, Virginia 22161
NTIS price codes—Printed Copy: A06; Microfiche A01

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. ADA 169506	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Water Quality Criteria for Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	5. TYPE OF REPORT & PERIOD COVERED Final Report 6. PERFORMING ORG. REPORT NUMBER ORNL-6178
7. AUTHOR(s) Elizabeth L. Etnier	8. CONTRACT OR GRANT NUMBER(s) 84 PP4845
9. PERFORMING ORGANIZATION NAME AND ADDRESS Chemical Effects Information Task Group Information Research and Analysis Section Oak Ridge National Laboratory, Oak Ridge, TN 37831	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick, Frederick, MD 21701-5010	12. REPORT DATE June 1986 13. NUMBER OF PAGES 123 pp.
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)	15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release Distribution unlimited	
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)	
18. SUPPLEMENTARY NOTES	
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) munition products, RDX, health effects, environmental effects, environmental fate, water quality criteria, aquatic toxicity, mammalian toxicity, metabolism	

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Health and environmental effects data were analyzed for RDX and a literature review presented. Information on the toxic effects of RDX on aquatic organisms is limited. From the data that are available, it appears that freshwater fish are more susceptible to RDX toxicity than freshwater invertebrates, having a range of LC_{50} values from 4.1 to 6.0 mg/L in 96-hr static tests, and 6.6 to 13 mg/L in 96-hr flow-through tests. (Continued)

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

20. ABSTRACT (Continued)

EC_{50} values (based on immobilization) of >15 mg/L in flow-through tests and >100 mg/L in static tests were reported for four freshwater invertebrate species. Bioconcentration of RDX in freshwater fish appears to be minimal, with values for edible tissue ranging from 1.4 to 1.7.

Chronic RDX intoxication in workers is characterized by epileptiform seizures (generalized convulsions) and unconsciousness. Seizures are followed by temporary amnesia, disorientation, and asthenia. No clinical information describing fatal cases of RDX poisoning is available.

Oral LD_{50} values reported in the literature for RDX range from 44 to 300 mg/kg in the rat. During a 2-yr feeding study with rats, the major toxic effects of RDX included anemia with secondary splenic lesions, hepatotoxicity, possible CNS involvement, and urogenital lesions. The authors report an NOEL under their study conditions of 0.3 mg/kg/day. In another 2-yr study evaluating the chronic toxicity and carcinogenicity of RDX in mice, the authors report an NOEL of 1.5 mg/kg/day. This study suggests the possibility of carcinogenicity of RDX. However, the absence of an adequate dose-response curve and a high mortality rate recorded at the highest concentration tested preclude the development of a carcinogenic-based risk assessment for humans. No evidence of genotoxicity or developmental/reproductive toxicity of RDX was found in the literature.

Insufficient data were available to calculate a water quality criterion for aquatic organisms using USEPA guidelines. Based on noncarcinogenic mammalian toxicity data, an ambient water quality criterion for the protection of human health and sensitive populations of 103 ug/L is proposed. It appears that this level of protection for humans will also adequately protect aquatic organisms and their uses.

AD
ORNL-6178

WATER QUALITY CRITERIA FOR HEXAHYDRO-1,3,5-TRINITRO-1,3,5-TRIAZINE (RDX)

FINAL REPORT

Elizabeth L. Etnier

Chemical Effects Information Task Group
Information Research and Analysis
Biology Division

JUNE 1986

SUPPORTED BY

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Date Published - June 1986

OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37831
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MARTIN MARIETTA ENERGY SYSTEMS, INC.
for the
U.S. DEPARTMENT OF ENERGY
Under Contract No. DE-AC05-84OR21400

EXECUTIVE SUMMARY

Hexahydro-1,3,5-trinitro-1,3,5-triazine (CAS 121-82-4) is a white crystalline high explosive extensively used by the military. It is commonly known as RDX (British code name for Research Department Explosive or Royal Demolition Explosive).

RDX has a very low solubility in water and in apolar organic solvents; it is readily soluble in polar organics such as acetone, dimethylsulfoxide (DMSO), and cyclohexanone.

The majority of available data indicate that RDX cannot be degraded when it is the sole source of carbon or if aerobic conditions are present. However, when exposed to anaerobic sediment-populations of bacteria and extra nutrients, RDX can be reduced or transformed within a few weeks. Physical degradation of RDX may occur by photolysis, hydrolysis, or volatilization. Hydrolysis and volatilization are not expected to significantly influence the environmental fate of RDX as they proceed very slowly.

The USEPA guidelines for estimating water quality criteria for aquatic organisms and their uses state that at a minimum, results of acute toxicity tests with freshwater families in at least eight different, specified, families be available before criteria can be calculated. Information on the toxic effects of RDX on aquatic organisms is limited. Thus the minimum data base requirements for derivation of a water quality criteria for aquatic animals are not satisfied. However, from the data that are available, it appears that freshwater fish are more susceptible to RDX toxicity than freshwater invertebrates, having a range of LC_{50} values from 4.1 to 6.0 mg/L in 96-hr static tests, and 6.6 to 13 mg/L in 96-hr flow-through tests. Although no definitive 24-, 48-, 72-, or 96-hr EC_{50} values (based on immobilization) are available for freshwater invertebrates, EC_{50} values of >15 mg/L in flow-through tests and >100 mg/L in static tests were reported for four invertebrate species. The USEPA guidelines accept greater than values from properly conducted tests for use in the estimation of a Final Acute Value (FAV). Although data were available for only six of the eight families required, an interim FAV of 5.1821 mg/L was generated based on the methodology presented in the USEPA guidelines.

Life cycle tests with P. promelas resulted in chronic values very similar to, but slightly lower than, the acute values reported for the same species. However, USEPA guidelines for water quality criteria specify the need for acute flow-through and chronic toxicity tests with measured concentrations for three species of organisms. This minimum data base is not satisfied for RDX, thus no Final Chronic Value and no acute-chronic ratio can be calculated.

Bioconcentration of RDX in freshwater fish appears to be minimal, with values for edible tissue ranging from 1.4 to 4.7 in the three species of fish tested. No maximum permissible tissue concentration is

available for RDX, nor are there any chronic wildlife feeding or field studies to estimate acceptable dietary intake. Therefore, no Final Residue Value can be calculated.

No definitive lower limit exists for plant toxicity, although 96-hr EC_{50} values (based on changes in cell density and chlorophyll a content) of >32 mg/L have been reported for four algal species. There is not sufficient data to arrive at a Final Plant Value.

No studies have been performed with saltwater vertebrates, invertebrates, or plants.

In humans and laboratory animals, RDX is slowly absorbed from the stomach after ingestion and also apparently from the lungs after inhalation; there is no clinical or experimental evidence of skin absorption. In laboratory animals it appears to be extensively metabolized in the liver, does not accumulate appreciably in any tissue, and is excreted primarily in the urine or exhaled as carbon dioxide (CO_2).

Chronic RDX intoxication among workers in the munitions industry has been documented in several studies, exposure occurring mainly from inhalation of fine particles; because RDX is not very lipid soluble, skin absorption is very unlikely. Chronic intoxication in workers is characterized by epileptiform seizures (generalized convulsions) and unconsciousness. Convulsions may appear without warning or be preceded by one or two days of insomnia, restlessness, and irritability. Seizures are followed by temporary amnesia, disorientation, and asthenia. A number of fatal cases suspected to be associated with RDX poisoning appear in occupational records from the 1940s, although no clinical information describing the cases is available.

One clinical report documenting the ingestion of RDX by a 14.5-kg, 3-yr-old child appears in the literature. Concentrations of RDX in blood, urine, and stool were quantitated over a 5-day period following onset of convulsions, and pharmacokinetic models utilized to estimate elimination time, half-life, and total ingested RDX.

Acute and chronic animal exposures to RDX produce toxicity similar to that seen in humans; central nervous system excitation is the most prominent effect of RDX. Other toxic manifestations include gasping and labored breathing. Pathological changes are generally nonspecific and consist of congestion in various organs, swelling and degeneration of renal tubular epithelium, fatty degeneration of the liver, and areas of hyaline degeneration in heart muscle. No pathological changes have been noted in the brain.

The oral LD_{50} values reported in the literature for RDX range from 44 to 300 mg/kg in the rat, indicating that RDX is moderately to highly toxic when administered to laboratory animals. There may be sex differences in response to RDX treatment.

Studies evaluating the neurobehavioral toxicity of acute RDX exposure in rats revealed a dose-related response in schedule-controlled

behavior, flavor-aversion conditioning, motor activity, and landing footspread at 12.5 mg/kg.

It has not been established whether RDX alone, or a metabolite of RDX, is the effective toxic agent causing convulsions and death in mammals. However, the short time period between intravenous injection of RDX to rats and dogs, and the onset of convulsions, suggests that the parent compound is responsible for the central nervous system effects.

Subchronic and chronic RDX toxicity tests have been performed in an effort to determine the mechanism of RDX toxicity and to establish no-effect dose levels. In an attempt to ascertain whether RDX was affecting the central nervous system (CNS), studies in the 1940s found that administration of nembutal, an antispasmodic drug, prevented convulsions and subsequent death, and that decerebrated rats exhibited no convulsive symptoms after intraperitoneal injection of a lethal dose of RDX.

Subchronic exposure to RDX was found to induce time- and dose-related, biphasic changes in brain monoamine oxidase, cholinesterase, and oxygen uptake. None of these effects were found in rats chronically dosed with 0.30 mg/kg/day, thus this concentration is reported as a no-observed-effect-level (NOEL).

During a 2-yr feeding study with rats, the major toxic effects of RDX included anemia with secondary splenic lesions, hepatotoxicity, possible CNS involvement, and urogenital lesions. Based on observations of suppurative inflammation of the prostate gland and increased levels of a hemosiderin-like pigment deposited in the spleen for rats administered 1.5 mg/kg/day or greater, the authors report an NOEL under their study conditions of 0.3 mg/kg/day.

In another study evaluating the chronic toxicity and carcinogenicity of RDX in mice, the major toxic effects observed after 2 yr included hepatotoxicity, possible CNS involvement, and testicular degeneration. A possible treatment-related elevation in serum triglyceride levels seen in female mice at 35 mg/kg/day, and elevated serum cholesterol levels seen in female mice at 35 mg/kg/day, and possibly 7 mg/kg/day, resulted in an NOEL of 1.5 mg/kg/day. This study suggests the possibility of carcinogenicity of RDX. However, the absence of an adequate dose-response curve and the high mortality rate recorded at the highest concentration tested, as well as the absence of any other supporting data, preclude the development of a carcinogenic-based risk assessment for humans. No evidence of genotoxicity or developmental/reproductive toxicity of RDX was found in the literature.

Since no conclusive evidence of carcinogenicity has been shown for RDX, and there are no available human epidemiological data, the calculation of a water quality criterion for the protection of human health comes from a 2-yr chronic feeding study with rats. As noted above, suppurative inflammation of the prostate gland and increased levels of a hemosiderin-like pigment deposited in the spleen for rats administered 1.5 mg/kg/day or greater was observed, and an NOEL of 0.3 mg/kg/day was reported.

Using the NOEL of 0.3 mg/L, and an uncertainty factor of 100, an acceptable daily intake for a 70-kg human is calculated to be 0.21 mg/day. The uncertainty factor of 100 was selected because the results are from long-term animal studies in which a well-defined LOAEL and NOEL exist. Using the methodology of the USEPA, an ambient water quality criterion for the protection of human health and sensitive populations of 103 µg/L is proposed. Based on the limited data available on the toxicity of RDX to aquatic organisms, it appears that this level of protection for humans will also adequately protect aquatic organisms and their uses.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Hexahydro-1,3,5-trinitro-1,3,5-triazine (CAS 121-82-4) is a white crystalline high explosive extensively used by the military. It is commonly known as RDX (British code name for Research Department Explosive or Royal Demolition Explosive). Other synonyms are: hexolite, cyclonite, hexogen, PBX (AF) 108, T4, cyclotrimethylenetrinitramine, trimethylenetrinitramine, and trinitrocyclotrimethylene triamine (Tatken and Lewis 1983).

During the 1960s, RDX was the third most important explosive from a tonnage viewpoint after trinitrotoluene (TNT) and nitrocellulose (Federoff and Sheffield 1966). Production of RDX from 1969 to 1971 averaged about 15 million pounds per month (Patterson et al. 1976). However, total production for 1984 was 15,946,243 pounds (Parmer 1986, personal communication). Current production of RDX is limited to Holston Army Ammunition Plant (AAP) (Kingsport, TN), which is operating at 10 to 20 percent capacity (Evans, personal communication, 1984). Handling/packing operations occur at several AAPs, including Holston, Louisiana (Shreveport, LA), Lone Star (Texarkana, TX), Iowa (Middletown, IA), and Milan (Milan, TN). Wastewaters resulting from the manufacture and loading of RDX may be discharged into the environment and represent a potential for aquatic pollution. Sediment deposits in settling ponds at these AAPs may also pose an environmental problem as seepage into the groundwater may occur.

The objectives of this report are to review the available data on the aquatic and human health effects of RDX and generate water quality criteria using the latest USEPA guidelines. Appendix A summarizes the USEPA methodology for deriving water quality criteria designed to protect aquatic life and its uses (Stephan et al. 1985), and Appendix B summarizes the USEPA methodology used to estimate water quality criteria designed to protect human health (USEPA 1980).

1.1 PHYSICAL AND CHEMICAL PROPERTIES

RDX has a very low solubility in water and in apolar organic solvents; it is readily soluble in polar organics such as acetone, dimethylsulfoxide (DMSO), and cyclohexanone (Urbanski et al. 1983). Spanggord (1977, as reported in Spanggord et al. 1980) gives the solubility of RDX in water as 44.7 mg/L at 18°C, very similar to the value of Sikka et al. (1980), 42.3 mg/L at 20°C. Patterson et al. (1976) report a solubility of 7.6 mg/L at 25°C, and 1.3 g/L at 83°C. Table 1 lists the solubility of RDX in various organic solvents.

Other physical and chemical properties of RDX are listed as follows (data from Lindner 1980, unless otherwise indicated):

Molecular formula: $C_3H_6N_6O_6$

TABLE 1. SOLUBILITY OF RDX[a] IN VARIOUS ORGANIC SOLVENTS (g/100 g)

Solvent	0°C	25°C	30°C	40°C	60°C	80°C	98°C
Methyl alcohol[b]	0.140	-	0.325	0.480	1.060	-	-
Ethyl alcohol[b]	0.040	-	0.155	0.235	0.575	-	-
Ethyl ether[b]	-	0.075	-	-	-	-	
Acetone[b]	4.18	-	8.38	10.34	15.27[c]	-	-
Benzene[b]	-	0.055	0.085	0.195	-	-	
Toluene[b]	0.016	0.025	0.050	0.125	0.295		
Carbon tetrachloride[b]	-	-	-	0.005	-	-	
Cyclohexanone[b]	-	12.7	-	-	-	27	
Dimethyl sulfoxide[d]	-	41	-	51	66	87	113
Dimethyl formamide[d]	-	37	-	45	58	76	96
N-Methylpyrrolidone[d]	-	40	-	47	58	72	84
Butyrolactone[d]	-	14	-	-	28	41	61

a. RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine.

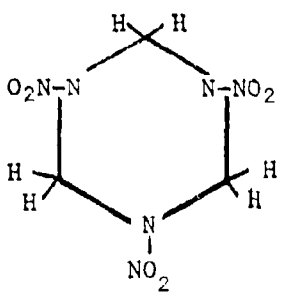
b. From Urbanski et al. (1983).

c. At 56.5°C.

d. From Gilbert (1980).

Molecular weight: 222.15 (Tatken and Lewis 1981)

Structural formula:



Elemental analysis: C, 16.22%; H, 2.72%; N, 37.83%; O, 43.22% (Windholz et al. 1983)

Color: White

Crystal density, g/cm^3: 1.83

Crystal form: Orthorhombic

Melting point: 204°C

Hardness, Mohs: 2.5

Oxygen balance, percent to CO_2: -22

Octanol-Water Partition Coefficient, Log P: ~ 0.87 (Banerjee et al. 1980); 0.78 (Atlantic Research Corporation 1979, as reported in Wentsel et al. 1979); 0.70 to 1.61 (as calculated by Wentsel et al. 1979, from data of Bentley et al. 1977)

Heat of formation, kJ/g: -0.277

Heat of fusion at 478.5 K: 8.5 kcal/mole (Roth 1980)

Heat of combustion, kJ/g: 9.46

Specific heat, J(g·K): 1.26; 0.398 cal/g°C at 20°C (Roth 1980)

Heat of vaporization, J/g: 490

Heat of sublimation, kcal/mole: 31.1 (Roth 1980)

Detonation products (calculated values, mole/mole RDX): 3.00 N_2, 3.00 H_2O, 1.49 CO_2, and 0.02 CO

Stability: Stored at 85°C for 10 months without perceptible deterioration

Dipole moment: ~7D (in highly polar solvents) (Roth 1980)

1.2 MANUFACTURING AND ANALYTICAL TECHNIQUES

Manufacture of RDX in the United States is exclusively by the rather simple Bachman Process (Lindner 1980). During this process hexamine is nitrated with a mixture of nitric acid, ammonium nitrate, acetic acid, and acetic anhydride to form 1 mole of RDX; 3 molecules of formaldehyde, liberated from the hexamine, react with the ammonium nitrate in the presence of acetic anhydride to form a second mole of RDX (Federoff and Sheffield 1966). In the Bachman process an 80-84 percent yield is obtained, about 10 percent of which is octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) (Lindner 1980). Achuthan and Mullick (1983) briefly review the fire, explosion, and toxic hazards of the chemicals used in the manufacture of RDX, including the potential hazard involved in the uncontrolled mixing of the chemicals.

Various techniques are available for the quantitative analysis of RDX. These include volumetric analysis, thin layer chromatography

(TLC), gas-liquid chromatography (GLC), high pressure liquid chromatography (HPLC), and single-sweep polarography (Sullivan et al. 1979). In general, volumetric analysis has not been modified to detect the low levels of RDX expected in the aquatic environment, although it is applicable to solid munition samples. Most workers have employed TLC, GLC, or HPLC techniques to environmental samples, the latter two resulting in lower detection limits than the former. Detection limits in the parts-per-billion range have been achieved with GLC (Hoffsommer, personal communication, as reported in Sullivan et al. 1979), although sample preparation is somewhat complicated and time consuming, entailing a risk of sample loss or destruction. A lower limit of 0.05 mg/L can be detected using HPLC, with an analysis time of about 15 min (Stilwell et al. 1977). Stilwell et al. (1977) used a Varian 8500 High Pressure Chromatograph equipped with a Dupont 837 variable multiwavelength ultraviolet (UV) detector, with the detection wavelength set at 230 nm. Whitnack (1976, as reported in Sullivan et al. 1979) developed a technique known as single-sweep polarography which will detect 0.05 mg/L RDX in a 2-mL aliquot of sample, with an analysis time of about 5 min. Sullivan et al. (1979) report that HPLC affords the best all-round analytical system for monitoring trace amounts of RDX in the aquatic environment. Regardless of the analytical technique utilized, due to the possibility of photolytic degradation of RDX, all samples must be stored in amber glass bottles with teflon-lined stoppers (Sullivan et al. 1979).

2. ENVIRONMENTAL EFFECTS AND FATE

2.1 ABIOTIC ENVIRONMENTAL EFFECTS

Little information was found in the literature for the effects of RDX processing effluents on habitat degradation. The majority of data were measurements of water quality parameters of load, assemble, and pack effluents containing a mixture of chemical compounds. RDX concentrations were measured at the Milan AAP (Envirodyne Engineers, Inc. 1980) and found to range from 0.4 to 110 µg/L in surface water and from 290 to 43,000 µg/g in stream sediments. Similar levels of RDX were also found in waste storage lagoons. High levels of RDX were often accompanied by high nitrate and sulfate concentrations (Envirodyne Engineers, Inc. 1980), although the relative impact of these elevated nitrate and sulfate values is hard to determine since no background levels were reported.

Stilwell et al. (1977) tested the waste effluents from two manufacturing areas of Holston AAP for munitions content and water quality (Table 2). The effluents from both areas contained elevated amounts of nitrogen species, phosphates, solids, and munition compounds. Chemical oxygen demand was higher than from the main Holston River (control). The relationship between RDX and these elevated parameters is impossible to determine. However, it is expected that the impacts of RDX on water quality would be moderated by waste treatment processes and by dilution (Ryon et al. 1984).

TABLE 2. SUMMARY OF WASTEWATER QUALITY DATA AT HOLSTON ARMY AMMUNITION PLANT[a]

Parameter (units)	Area A[b]	Area B[c]	Holston River (Control)
Chemical oxygen demand (ppm)	4,058	314	58
Biological oxygen demand (ppm)	2,798	191	ND[d]
pH	4.3	7.3	ND
Ammonia-N (ppm)	102.3	13.3	ND
Total Kjeldahl nitrogen (ppm)	126.8	22.7	ND
Phosphates (ppm)	420	24	ND
Nitrates (ppm)	38.9	43.9	ND
Nitrites (ppm)	40	1	ND
Total solids (ppm)	2,002	426	ND
HMX[e] (ppm)	3.59	0.77	<0.05
RDX[f] (ppm)	0.88	0.80	<0.05
TNT[g] (ppm)	<0.05	<0.05	<0.05

a. Adapted from Stilwell et al. 1977.

b. Area A effluent is composed of ten wastewater streams from acetic acid concentrators, steam generators, and filter plants.

c. Area B effluent is composed of four wastewater streams from the composition B production lines and the continuous RDX production line.

d. ND = not determined.

e. HMX = 1,3,5,7-tetrahydro-1,3,5,7-tetracyclooctane.

f. RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine.

g. TNT = 2,4,6-trinitrotoluene.

2.2 ENVIRONMENTAL FATE

2.2.1 Migration

Migration of RDX in soils was studied by Hale et al. (1979) utilizing cylinders (91 cm diameter by 152 cm deep) to withdraw intact soil columns which were spiked with RDX at the surface, planted with grass, and irrigated regularly in a controlled greenhouse environment. This study was supplemented with ^{14}C-labelled RDX using smaller lysimeters (5 cm by 61 cm) in an attempt to further define the migration of RDX in soils. Four soil types were chosen to represent a range of pH, texture, and organic matter typical of U.S. soils. Levels of RDX in water leachates were found to be less than 0.5 ppm (the level of detection) in both the main study and the ^{14}C study during the entire period. After 26 weeks following treatment, the concentration of RDX in soil samples collected at all depths in all four soils was less than 1 percent of that found at the 0-10 cm depth. Soil types did not seem to have an effect on the specific values. In the ^{14}C-labelled RDX studies, there was a significant (level of significance not reported) evolution of $^{14}CO_2$, indicating chemical or biological degradation of RDX near the surface of the soil. There was also some downward movement of labelled RDX through the soil columns, but, in both cases, the amount of RDX or transformation products was too low to be quantified.

2.2.2 Biological Degradation

The majority of available data indicate that RDX is resistant to degradation when it is the sole source of carbon or if aerobic conditions are present (Osmon and Klausmeier 1972; Hoffsommer et al. 1978; Spanggord et al. 1980; McCormick et al. 1981). However, when exposed to anaerobic sediment-populations of bacteria and extra nutrients, RDX can be reduced or transformed within 2 to 38 days (Natick 1980, as reported in Isbister 1980; Sikka et al. 1980; Spanggord et al. 1980; McCormick et al. 1981). Several species of bacteria that degrade RDX have been identified. Pseudomonas spp. and Alcaligenes spp. in an activated sludge reactor system were able to remove 42 percent of the RDX from HAAP area B wastes (Green 1972, as reported in Sullivan et al. 1979). Soli (1973) reported 97 percent of a 20 mg/L solution of RDX to be degraded in five days under anaerobic conditions, using a mixed population of purple photosynthetic bacteria in the families Thiorhodaceae and Athiorhodaceae. Yang et al. (1983) report >90 percent reduction of 40 to 60 mg/L RDX in one to three days by three strains of Corynebacterium isolated from soil long polluted with RDX. The medium contained a 0.1 percent carbon source at a pH of 6 to 7.5. The description does not indicate whether aerobic or anaerobic conditions prevailed.

RDX transformation products have been identified including formaldehyde, methanol, hydrazine, trinitrosotriazine, and a series of RDX-nitroso reduction products (Natick 1980, as reported in Isbister et al. 1980; McCormick et al. 1981). Cleavage of the ring structure in ^{14}C-RDX was indicated in soil composting studies (Hale et al. 1979) and in anaerobic sediments mixed with water (McCormick et al. 1981). The study by McCormick et al. described potential pathways for the anaerobic

degradation of RDX (Figure 1): RDX is sequentially reduced to its mono-, di-, and tri-nitroso analogs (compounds 2, 3, and 4 in Figure 1), each of which undergoes further reduction of a nitroso group to form a triazine (compounds 5, 6, and 7). Hydrolytic cleavage of these compounds, followed by rearrangement and further reduction of the fragments, gives rise to the end products, compounds 8, 9, 10, and 11. Compounds 8 and 9 are further reduced to yield formaldehyde and hydrazine (compounds 12 and 15 in Figure 2). Under strongly reducing conditions, formaldehyde is reduced to methanol (Figure 2). Compounds 10 and 10a (Figure 1) follow the reduction pathway followed by compound 13 (Figure 2). McCormick et al. (1981) propose that compound 11 (Figure 1) undergoes sequential reduction to form dimethylhydrazines. In a study investigating the use of composting for degrading high concentrations of RDX, Isbister et al. (1984) found that RDX was completely degraded with evolution of CO_2, and with RDX breakdown products (unidentified) apparently assimilated into the microbial mass as quickly as they were produced.

Spanggord et al. (1980) studied microbial degradation under various environmental conditions in the laboratory. Natural aerobic biodegradation of RDX was found to proceed slowly. In aerobic conditions, degradation of RDX did not occur after 90 days of incubation with water from the Holston River. Addition of sediment did produce a decrease from 10 ppm to 4 ppm after 36 days (perhaps due to sorption), but two more weeks of incubation produced no further degradation. However, in anaerobic conditions with the presence of extra organic materials, significant degradation (level of significance not reported) occurred, with a 30-ppm solution of RDX reduced to <0.1 ppm after 10 days. This indicates that in most stream systems (aerobic conditions), persistence of RDX would be fairly lengthy. In lake-type aquatic situations (anaerobic conditions), biological degradation of RDX could occur to a greater extent (Spanggord et al. 1980).

2.2.3 Physical Degradation

The primary physical mechanism that degrades RDX in aqueous solutions is photolysis. The range of UV wavelengths that causes photolytic reactions with RDX is generally between 240 and 350 nm.

Andrews and Osmon (1976) found that at 240-260 nm, RDX was totally degraded after 312 hr. In a maximum exposure situation, 45 ppm of RDX was reduced without any TLC-detectable products after 2 hr. Photolysis of RDX exposed to UV light around 230 nm resulted in about a 45 percent reduction of RDX in less than 5 hr (Sikka et al. 1980). Nitrate and nitrite concentrations were found to be increased during the irradiation.

Kubose and Hoffsommer (1977) investigated the use of photolysis to degrade RDX in aqueous solution. They initially used a full spectral array of 220 to 1367 nm and found that RDX in aqueous solution was rapidly destroyed; limiting the wavelengths to greater than 280 nm resulted in a greatly reduced disappearance of RDX. The rapid photolytic destruction of RDX at less than 280 nm was reproduced in flow systems of varying depth and rate of flow. The authors (Kubose and

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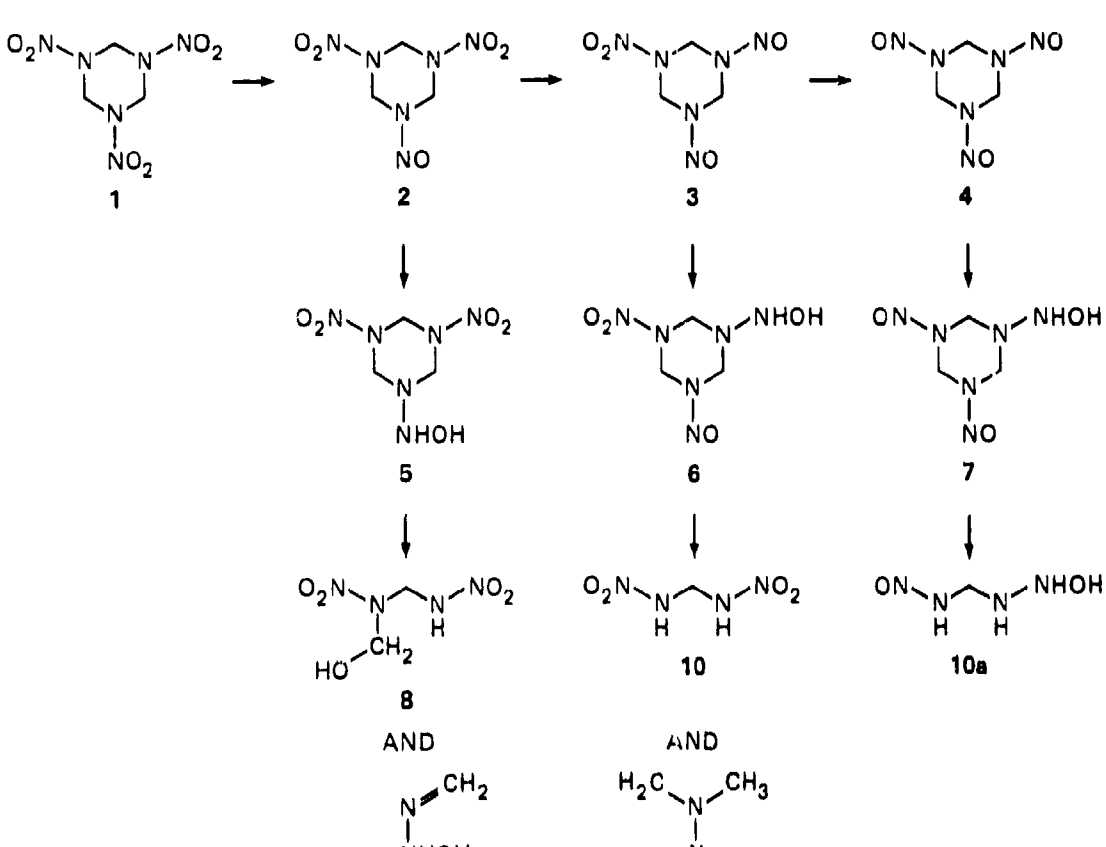

Figure 1. Proposed anaerobic degradation pathway of RDX. Compounds are: 1 = RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine); 2 = MNX (hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine); 3 = DNX (hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine); 2,4 = TNX (hexahydro-1,3,5-trinitroso-1,3,5-triazine); 5 = 1-hydroxylamino-3,5-dinitro-1,3,5-triazine; 6 = 1-hydroxylamino-3-nitroso-5-nitro-1,3,5-triazine; 7 = hydroxylamino-3,5-dinitroso-1,3,5-triazine; 8 = N-hydroxymethylmethylene-dinitramine; 9 = N-hydroxymethylhydrazine; 10 = N-hydroxylamino-N'-nitromethylenediamine; 10a = N-hydroxyl-amino-N'-nitroso-methylenediamine; and 11 = dimethylnitrosamine radical.

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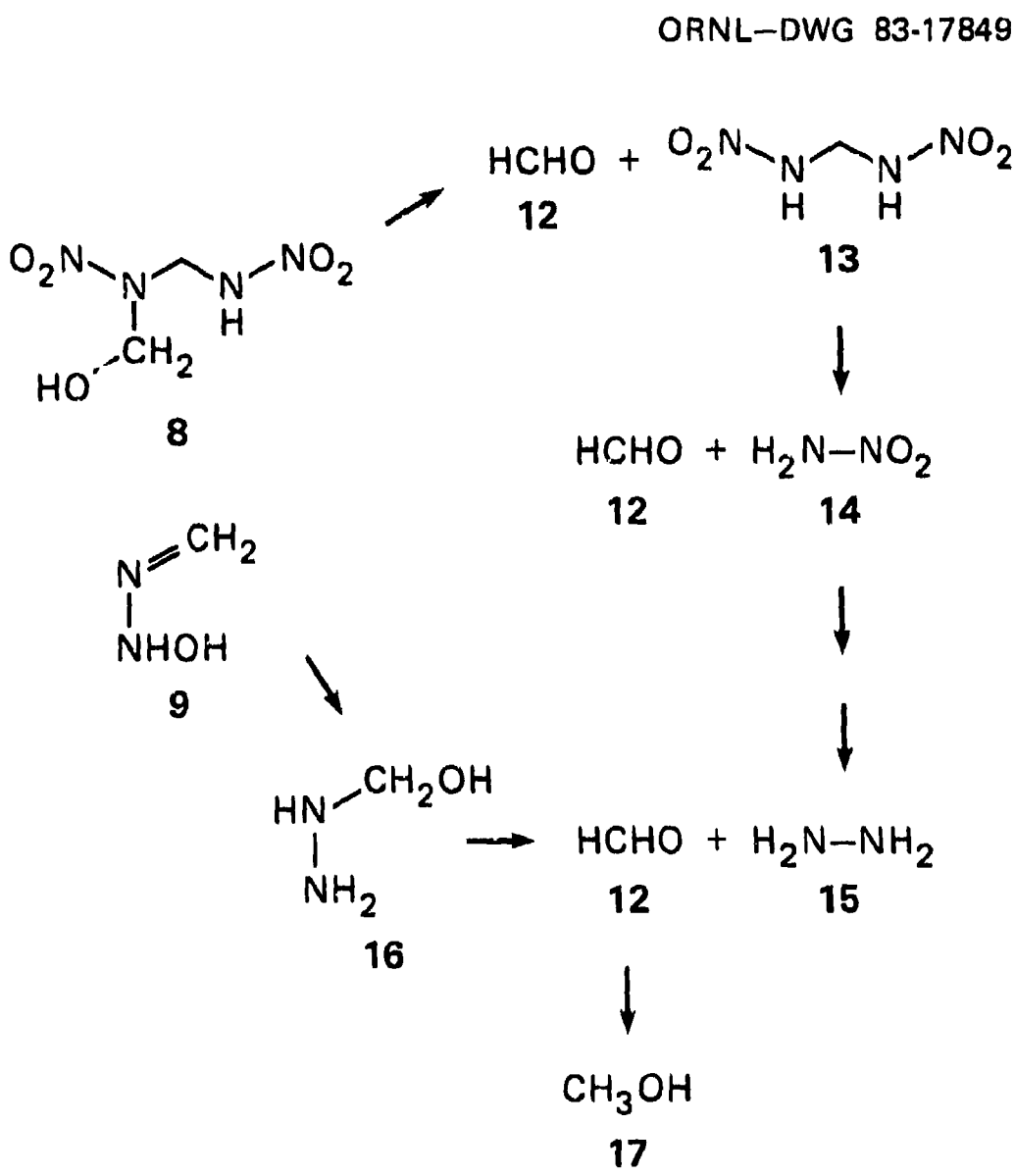

Figure 2. Final stages of proposed anaerobic degradation pathway of RDX-derived compounds. Compounds are: 8 = N-hydroxymethyl-methylenedinitramine; 9 = N-hydroxymethylene-hydrazone; 12 = HCHO; 13 = methylenedinitramine; 14 = nitramide; 15 = hydrazine; 16 = hydroxymethylhydrazine; and 17 = methanol. From McCormick et al. 1981.

Source: Reprinted from Applied and Environmental Microbiology 42(5):817-823 (1981), by McCormick et al., with permission of the American Society for Microbiology.

Hoffsommer 1977) found that the percent of RDX removed appeared to be independent of depth (up to 3 cm), indicating that the degradation products do not interfere by strongly absorbing the wavelengths responsible for RDX degradation. It was also shown that the pH of the distilled water solution did not affect the rate of photolysis.

Smetana and Bulusu (1977) also studied the degradation of aqueous solutions of RDX by UV photolysis at 254, 300, and 350 nm, as well as by ozonolysis alone, and by a combination of 350 nm and ozone. They achieved very rapid rates of disappearance of RDX (less than 1 hr) at the 254-nm wavelength, and found that the higher wavelengths studied were much less effective, destroying only about half of the RDX in solution after 10 hr. Exposure of solid RDX to 36\ nm failed to cause any degradation, and no gas was evolved in over 10 hr. Relatively high concentrations of ozone were much slower in degrading RDX, only about 50 percent of the RDX being degraded in 10 hr. An apparent synergistic effect between ozone and 350 nm was observed, with the combination causing about 95 percent degradation of RDX in 5 hr (Smetana and Bulusu 1977).

Photolysis of RDX in distilled water and in natural water samples followed first-order kinetics when photolyzed in sunlight or at 313 nm (Spanggord et al. 1980). Sunlight photolysis rates in the laboratory were similar for the two water types, and estimated half-lives ranged from 9 to 14 days. The photolytic half-life on a sunny day in the Holston River was estimated by Spanggord et al. to be thre days, indicating that RDX discharged from the Holston AAP would remain in the river for a long distance downstream. Spanggord et al. calculated the half-life for RDX photolysis as a function of latitude and season (Table 3). No photodegradation products were identified during HPLC analysis of the photolyzed solutions; the authors particularly attempted to identify N-nitroso compounds. Formaldehyde, nitrite, and nitrate were identified as byproducts of photolysis using gas chromatography.

Wentsel et al. (1979), using the data of Spanggord et al. (1980) and Sikka et al. (1980), indicate that RDX is removed from solution at a rate between 0.11 to 0.27 ppm/hr. Spanggord et al. (1978) studied the photolysis of TNT and RDX together and found an apparent antagonism, with RDX being reduced at a rate of only 0.03 ppm/hr.

The degradation pathway for RDX when exposed to UV light was also discussed by Kubose and Hoffsommer (1977). They found two possible routes of decomposition depending on the UV wavelength. At wavelengths greater than 280 nm, the principal step is the homolytic cleavage of the nitramine bond to give an azayl radical and NO_2. When this occurs in acidic conditions, NO is formed and reacts with the azayl radical to form a mono-nitroso analog of RDX (they proposed 1-nitroso-3,5-dinitro-1,3,5-triazacyclohexane). When the wavelengths are near 220 nm, the primary step is cleavage of the N-O bond of the nitro group to form the nitroso analog directly. This reaction is independent of pH. Other metabolites formed, and their mole ratios to RDX, are given in Table 4. The presence of formaldehyde, ammonia, and the other gases indicates that the ring is broken in the final degradation steps. Smetana and

TABLE 3. ANNUAL VARIATION OF PHOTOLYSIS HALF-LIFE[a] OF RDX[b] IN SUNLIGHT IN DISTILLED WATER[c]

Season	RDX[c] (N latitude)		
	20°	40°	50°
Summer	1.1	1.2	1.3
Fall	1.4	2.6	4.4
Winter	1.8	5.0	12.5
Spring	1.2	1.5	2.0

a. Half-lives are in 24-hr days (10 hr sunlight).

b. RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine.

c. Adapted from Spanggord et al. 1980.

TABLE 4. MOLE RATIO OF PRODUCTS TO PHOTOLYZED RDX[a,b]

Wavelength (λ)	$\frac{NO_3^-}{RDX}$	$\frac{NO_2^-}{RDX}$	$\frac{CH_2}{RDX}$	$\frac{NH_3}{RDX}$	$\frac{N_2O}{RDX}$	$\frac{N_2}{RDX}$
>220 nm	trace	2.4	0.8	0.6	0.05-0.1	0.1-0.2
>280 nm	0.7	2.0	0.6	0.7	-	-

a. RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine.

b. Adapted from Kubose and Hoffsommer 1977.

Bulusu (1977) also found these final metabolic products. Spanggord et al. (1980) identified many of these products including formaldehyde and other gases. However, they were unable to confirm the presence of the nitroso analog found by Kubose and Hoffsommer (1977).

Other physical methods of degradation include hydrolysis, volatilization, and thermal decomposition. Spanggord et al. (1980) estimate a half-life for RDX volatilization of 9×10^6 days, and suggest that volatilization should not be a significant environmental fate for RDX. Sikka et al. (1980) found hydrolysis to be a slow process, with virtually none occurring under acidic conditions over a 12-day period. Under basic conditions, they achieved a 27 percent reduction of RDX by hydrolysis in three weeks. Hydrolysis in sea water was also much slower than photolysis and only reduced about 12 percent of a 56 mg/L solution of RDX after 112 days (Hoffsommer and Rosen 1973). Thermal decomposition

of RDX was quite similar to photolysis, reducing 93 percent of an RDX solution (in an unspecified length of time) (Smetana and Bulusu 1977). The thermal decompostion products were similar, although in different ratios, to those obtained from photolysis and/or ozonolysis, with CO_2, N_2O, N_2, NO, CO, HCN, HC_2O, and H_2O found.

2.2.4 Sediment Adsorption

Sikka et al. (1980) measured the adsorption of RDX on three soil types, and reported partition coefficients of 0.80 for sandy loam, 3.06 for clay loam, and 4.15 for organic muck. Even with such low adsorption, steady-state levels of RDX between 30 to 40 ppm were reported for organic and clay sediments (Sikka et al. 1980). Sediment concentrations in the parts per million range might be expected in natural environments containing RDX (Wentsel et al. 1979).

Sediment adsorption partition coefficients were estimated by Spanggord et al. (1980) to be extemely low (less than six in all studies), indicating that sediment sorption will not lead to a significant RDX loss in the aquatic environment. Hale et al. (1977) likewise estimated partition coefficients for four soils representing a range of pH, texture, and organic matter content typically found in soils throughout the United States. Soils selected were a Brookston silty clay loam, a Bennington silt loam, a Genessee silt loam, and a Princeton sandy loam. Partition coefficients reported for the soils were 7.8, 1.8, 6.4, and 0.2, respectively. Hale et al. suggest that this adsorption is irreversible, as significant amounts of RDX were not recovered in either water or soil extracts.

2.3 SUMMARY

The majority of available data indicate that RDX is resistant to degradation when it is the sole source of carbon or if aerobic conditions are present. However, when exposed to anaerobic sediment-populations of bacteria and extra nutrients, RDX can be reduced or transformed within a few weeks. RDX transformation products have been identified and include formaldehyde, methanol, hydrazine, trinitrosotriazine, and a series of RDX-nitroso reduction products. Cleavage of the ring structure in ^{14}C-RDX was indicated in soil composting studies and in anaerobic sediments mixed with water.

Natural aerobic biodegradation of RDX was found to proceed slowly. In aerobic conditions, degradation of RDX did not occur after 90 days of incubation with water from the Holston River. However, in anaerobic conditions with the presence of extra organic materials, significant degradation occurred within 2 to 38 days.. This indicates that in most stream systems (aerobic conditions), persistence of RDX would be fairly lengthy. In lake-type aquatic situations (anaerobic conditions), biological degradation of RDX could occur to a greater extent.

The primary physical mechanism that degrades RDX in aqueous solutions is photolysis. The range of UV wavelengths that causes photolytic

reactions with RDX is generally between 220 and 350 nm. Photolysis of RDX in distilled water and in natural water samples followed first-order kinetics when photolyzed in sunlight or at 313 nm. Sunlight photolysis rates in the laboratory were similar for the two water types, and estimated half-lives ranged from 9 to 14 days.

The degradation pathway for RDX when exposed to UV light involves two possible routes of decomposition depending on the UV wavelengths. At wavelengths greater than 280 nm, the principal step is the homolytic cleavage of the nitramine bond to give an azayl radical and NO_2. When the wavelengths are near 220 nm, the primary step is cleavage of the N-O bond of the nitro group to form the nitroso analog directly. Other physical methods of degradation include hydrolysis and volatilization, which proceed slowly, and are not expected to be a significant environmental fate for RDX.

Sediment adsorption partition coefficients were estimated to be extemely low (less than six in all studies), indicating that sediment sorption will not lead to a significant RDX loss in the aquatic environment.

3. AQUATIC TOXICOLOGY

3.1 ACUTE TOXICITY TO ANIMALS

Data available for calculating a water quality criteria for RDX do not meet all the requirements specified by the USEPA guidelines (Stephan et al. 1985); i.e., only six of the eight families of aquatic test animals required by the USEPA guidelines (see Appendix A) have been used in acute tests. However, since the available data generated by toxicity tests are uniform in their assessment of the degree of toxicity of RDX, information on the toxic effects of RDX on aquatic organisms is presented here. Table 5 summarizes the appropriate acute toxicity values used in the calculation of water quality criteria for the protection of aquatic organisms. Details of the aquatic toxicity studies are discussed below.

3.1.1 Aquatic Invertebrates

Bentley et al. (1977) tested four invertebrate species for acute toxic effects from RDX dissolved in DMSO. They used the waterflea Daphnia magna, the isopod Asellus militaris, the amphipod Gammarus fasciatus, and the midge larva Chironomus tentans in static tests of 24- and 48-hr duration at 20°C and 35 ppm hardness. The effective concentration causing a 50 percent response (EC_{50}), based on immobilization, was determined to be >100 mg/L for all species over both durations. The use of this value to quantitate RDX toxicity to macroinvertebrates may not be applicable in situations where RDX is not admixed with organic solvents which might increase its solubility as does DMSO. The authors did not present data comparing nominal to measured values actually occurring in the test water at these high concentrations. However, data presented

TABLE 5. ACUTE TESTS FOR IMMOBILIZATION[a] OR MORTALITY[b] OF AQUATIC SPECIES FOLLOWING EXPOSURE TO RDX[c,d]

Test Species	Test Method	Test Duration	LC50/EC50 (mg/L)	Genus Mean Acute Value (mg/L)
Arthropoda				
Crustacea				
Daphnidae				
Daphnia magna[a,e]	S[f]	48 hr	>100	
Daphnia magna[a,e]	FT[g]	48 hr	>15	>15
Gammaridae				
Gammarus fasciatus[a,h]	S	48 hr	>100	>100
Asellidae				
Asellus militaris[a,h]	S	48 hr	>100	>100
Insecta				
Chironomidae				
Chironomus tentans[a,i]	S	48 hr	>100	
Chironomus tentans[a,i]	FT	48 hr	>15	>15
Osteichthyes				
Centrarchidae				
Lepomis macrochirus[b,j]	S	96 hr	6.0 (5.4-6.5)[k]	
Lepomis macrochirus[b,j]	FT	96 hr	7.6 (5.6-10)	6.75
Salmonidae				
Salmo gairdneri[b,l]	S	96 hr	6.4 (5.4-7.4)	6.4
Ictaluridae				
Ictalurus punctatus[b,m]	S	96 hr	4.1 (3.5-4.9)	
Ictalurus punctatus[b,m]	FT	96 hr	13 (8.8-20)	7.3
Cyprinidae				
Pimephales promelas[b,n]	S	96 hr	4.5 (3.7-5.4)[o]	
Pimephales promelas[b,n]	S	96 hr	5.8 (4.7-7.2)	
Pimephales promelas[b,n]	FT	96 hr	6.6 (5.0-8.7)	5.6

a. Immobilization tests were designed to give EC_{50} values.

b. Mortality tests were designed to give LC_{50} values.

c. RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine.

d. From Bentley et al. 1977.

e. Test animals were 0 to 24 hr old at start of test.

f. S = static test.

g. FT = flow-through test.

h. Test animals were juveniles at start of test.

i. Test animals were second or third instars at start of test.

j. Test animals had a mean weight of 1.0 ± 0.3 g and a standard length of 35 ± 6 mm at start of test.

k. Values in parentheses indicate the 95% confidence interval.

l. Test animals had a mean weight of 0.9 ± 0.3 g and a standard length of 43 ± 4 mm at start of test.

m. Test animals had a mean weight of 1.2 ± 0.5 g and a standard length of 57 ± 11 mm at start of test.

n. Test animals had a mean weight of 1.0 ± 0.4 g and a standard length of 43 ± 8 mm at start of test.

o. From Liu et al. 1983.

comparing nominal to measured concentrations during flow-through tests at 0.13 to 5.0 mg RDX/L indicated that measured nearly equaled, or exceeded, nominal values at the levels tested.

A flow-through assay was also performed using midges and waterfleas. The flow rate in these assays was 4 L/day and the other test variables were similar to those in the static tests. No effects were found in these tests, with EC_{50} values estimated to be >15 mg/L (the highest nominal concentration used).

In a field survey of the aquatic systems affected by the Milan AAP, Huff et al. (1975) were unable to attribute any significant effect (level of significance not reported) on the zooplankton and macroinvertebrate populations from the RDX concentrations in the water. The authors note that the low abundance of aquatic organisms in the study area made a correlation between RDX concentration and effect on the environment virtually impossible. The low numbers of recorded organisms were due partly to poor habitat and partly to fluctuating flows and high suspended sediments. No other field data were found for RDX.

3.1.2 Fish

The toxicity of RDX to four species of fish was determined by Bentley et al. (1977) in static acute tests of 24-, 48-, and 96-hr durations. They exposed bluegill sunfish (Lepomis macrochirus), fathead minnows (Pimephales promelas), rainbow trout (Salmo gairdneri), and channel catfish (Ictalurus punctatus) to RDX dissolved in DMSO and reported that the LC_{50} values for RDX ranged from 4.1 to 14 mg/L (Table 6). Channel catfish were the most sensitive of the species tested. A flow-through test (nominal concentrations) was performed using bluegill sunfish, rainbow trout, and channel catfish with a flow rate of 5 L/hr and temperature of 21°C. The LC_{50} values from these tests were in the same range as those of the static tests, except for the 96-hr value for channel catfish, which was approximately 2.5 times greater (Table 7). Bentley et al. (1977) also tested various life stages of fathead minnows for acute toxicity using static test conditions. They found that acute toxicity of RDX to various life stages varied considerably (Table 8). The 7-day posthatching stage appeared to be the most sensitive, having an LC_{50} of 3.8 mg/L after 96 hr. The 30- and 60-day old fry exhibited similar sensitivities, with the latter slightly less tolerant. To verify that test conditions were not a significant factor, Bentley et al. varied temperature from 15 to 25°C, pH from 6 to 8, and hardness (in mg/L $CaCO_3$) from 35 to 250 in a static test using bluegill sunfish (Table 9). The LC_{50} values were generally equivalent with some decrease in toxicity seen at the lowest temperature. A similar test of the effect of aging the test RDX solution for periods of 12, 24, 48, and 96 hr prior to bioassay produced nearly identical LC_{50} values for bluegill sunfish for all time periods (4.8, 5.1, 4.8, and 4.8 mg/L, respectively), indicating that concentrations of RDX are stable during the duration of the standard 96-hr toxicity test (Bentley et al. 1977).

Liu et al. (1983) present 96-hr LC_{50} values obtained in static tests with fathead minnows using RDX alone (Table 5), and in various mixtures

TABLE 6. ACUTE TOXICITY VALUES FOR RDX[a] IN FISHES DETERMINED DURING STATIC TOXICITY TESTS[b]

Species	LC_{50} (mg/L)		
	24 hr	48 hr	96 hr
Lepomis macrochirus (bluegill)	14 (12-17)[c]	8.5 (7.5-9.5)	6.0 (5.-6.5)
Salmo gairdneri (rainbow trout)	9.4 (8.5-10)	7.0 (6.3-7.7)	6.4 (5.4-7.4)
Ictalurus punctatus (channel catfish)	7.5 (0.7-8.5)	6.0 (5.3-6.9)	4.1 (3.5-4.9)
Pimephales promelas (fathead minnow)	10 (7.4-14)	5.8 (6.9-12)	5.8 (4.7-7.2)

a. RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine.

b. Adapted from Bentley et al. 1977.

c. Values in parentheses indicate the 95% confidence interval.

TABLE 7. ACUTE TOXICITY OF RDX[a] TO FISHES DURING DYNAMIC TOXICITY TESTS (NOMINAL CONCENTRATION)[b]

Species	LC_{50} (mg/L)		
	24 hr	96 hr	Incipient[c]
Lepomis macrochirus (bluegill)	>10	7.6 (5.6-10)[d]	6.4 (5.3-7.8)
Ictalurus punctatus (channel catfish)	>10	13 (8.8-20)	11 (9.1-13)
Pimephales promelas (fathead minnow)	>10	6.6 (5.0-8.7)	5.2 (4.3-6.4)

a. RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine.

b. Adapted from Bentley et al. 1977.

c. Incipient LC_{50} is the nominal concentration which caused 50% mortality with no additional response (<10%) during the final 48 hr of exposure (estimated after 264 hr).

d. Values in parentheses indicate the 95% confidence interval.

TABLE 8. ACUTE TOXICITY OF RDX[a] TO SELECTED LIFE STAGES OF THE FATHEAD MINNOW (Pimephales promelas) AS DETERMINED DURING STATIC TOXICITY TESTS[b]

Life Stage	LC_{50} (mg/L)			
	24 hr	48 hr	96 hr	144 hr
Eggs	>100	>100	>100	>100
1-hr Posthatch	>100	>100	43 (27-69)[d]	-[c]
7-day Posthatch	32	18 (13-24)	3.8 (3.0-5.0)	-
30-day Posthatch	18 (13-24)	16 (13-19)	16 (13-19)	-
60-day Posthatch	11 (6.1-21)	11 (5.9-21)	11 (5.9-21)	-

a. RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine.

b. Adapted from Bentley et al. 1977.

c. Tests with fry were 96 hr in duration.

d. Values in parentheses indicate the 95% confidence interval.

TABLE 9. ACUTE TOXICITY OF RDX[a] TO BLUEGILL (Lepomis macrochirus) UNDER VARYING CONDITIONS OF WATER QUALITY DURING STATIC TOXICITY TESTS[b]

Temperature (°C)	pH	Hardness (mg/L $CaCO_3$)	96-hr LC_{50} (mg/L)
15	7.0	35	8.4 (6.0-11)[c]
20	7.0	35	5.1 (3.9-6.7)
25	7.0	35	4.1 (3.0-5.6)
20	7.0	35	3.8 (2.0-7.1)
20	7.0	100	5.3 (4.1-5.8)
20	7.0	250	3.9 (2.1-7.3)
20	6.0	35	3.6 (1.9-6.6)
20	7.0	35	3.7 (2.0-6.9)
20	8.0	35	3.9 (2.1-7.3)

a. RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine.

b. Adapted from Bentley et al. 1977.

c. Values in parentheses indicate the 95% confidence interval.

with 2,4,6-trinitrotoluene (TNT). They found that TNT and RDX act antagonistically in ratios of 1:1, 1.6:1, and 1:3, causing the mixtures to be less toxic than the sum of their two toxicities. At a ratio of three parts TNT to one part RDX, a slight synergism in toxicities was noted; i.e., the LC_{50} was 1.43 times lower than the theoretical additive LC_{50} for the two compounds (Liu et al. 1983).

An evaluation of the toxic effects on fathead minnows from exposure to wastewater from an AAP producing HMX and RDX explosives was performed by Stilwell et al. (1977). They used various dilutions of the waste effluents that contained both explosives at concentrations no greater than 6.0 ppm (RDX concentrations ranged from <0.05 to 5.17 ppm), and exposed the fish for 96 hr in a static assay. Some of the solutions failed to produce any lethality. RDX was not significantly correlated (at the 95 percent level) with any toxicity levels or water quality parameters (except total solids). Partial correlation coefficients were calculated by holding certain variables constant, and indicated no positive correlation between the LC_{50} values and the RDX content of the water when the controlled variable was biological or chemical oxygen demand. However, when ammonia or total nitrogen were the controlled variables, the correlation between RDX and toxicity was significant at the 99 percent level or greater. Stilwell et al. (1977) state that this indicates that RDX may have been found to be significantly related to toxicity if the various forms of nitrogen had been held at a fixed level in all the effluent samples.

In a field survey of the aquatic systems impacted by the Milan AAP, Huff et al. (1975) were unable to attribute any significant effect on the fish populations to the RDX concentrations in the water. However, the low abundance of aquatic organisms primarily due to fluctuating flows, heavy suspended sediments, and lack of suitable habitat, precluded any firm conclusions from the study. No other field data on fishes were found for RDX.

3.2 CHRONIC TOXICITY TO ANIMALS

Bentley et al. (1977) tested Daphnia magna in a chronic study using flow-through conditions. Mean measured RDX concentrations ranged from 1.4 to 20 mg/L, and were found to have no effect on survival throughout the first generation exposure period (21 days). Mean number of young produced per parthenogenic female exposed to RDX between days 7 and 14 was found to be significantly ($\alpha = 0.05$) reduced at 4.8, 9.5, and 20 mg/L. At the lower concentrations (1.4 and 2.2 mg/L) from days 7 to 14, and at all concentrations from day 14 to 21 of the first generation study and from days 35 to 42 of the second generation study, survival and mean number of young per parthenogenic female were found to be comparable to controls. The authors suggested that the significant reduction in young produced between days 7 and 14 may have been due to a higher (unspecified) concentration of RDX at these time intervals. In the studies of Bentley et al (1977), the mean percent survival of control daphnids during the first and second generations ranged from 78 to 82, and the mean young produced per parthenogenic control female in the first and second generation ranged from 14 to 22. These low results for

control populations would not meet current standards for aquatic toxicity testing. For example, the Organization for Economic Cooperation and Development Guidelines (OECD 1981) for testing of chemicals require that mortality of controls should not exceed 20 percent at the end of the test, and that the average cumulative number of young per female in the controls after three broods should be greater than 20. The USEPA (1985) has similar requirements for mortality in controls, but requires that number of young per parthenogenic female be no less than 60 during a 21-day test. Based on the problems Bentley et al. (1977) describe in maintaining RDX concentrations in the test chambers during days 7 to 14 of the test, and the fact that the control data are below currently acceptable standards, the use of this data to derive a final chronic toxicity value is questionable.

In a one-generation study using flow-through conditions, the chronic exposure of Chironomus tentans to RDX concentrations ranging from 1.3 to 21 mg/L had no statistically significant ($\alpha = 0.05$) effects on larvae, pupae, or adult survival, or adult emergence (Bentley et al. 1977). However, the average number of eggs produced per adult was greatly reduced from controls, with no fertile eggs at the 1.3 and 4.0 mg/L concentrations, and no eggs produced at all at the 10 mg/L concentration. During a second-generation exposure period, no significant ($\alpha = 0.05$) differences from controls were observed in percent survival of pupae at all levels, and survival of adults at the 2.2, 10, and 21 mg/L RDX exposure levels. Significant ($\alpha = 0.05$) reduction in second-generation adult emergence seen at 2.2 mg/L, in larval survival at all concentrations, and in adult survival at 1.3 and 4.0 mg/L was not conclusively related to RDX exposures, as second-generation studies at 1.3, 4.0, and 10.0 mg/L were initiated with control eggs (Bentley et al. 1977). The absence of toxic effects on survival and emergence during the first-generation exposures indicated to the authors that the effects seen in the second-generation study were not related to RDX exposure.

Critical life stage tests studied the effects of 30-day continuous exposure to RDX on the eggs and fry of channel catfish and fathead minnows (Bentley et al 1977). Equipment malfunctions in the channel catfish studies make the results of that study suspect, and so they will not be presented. No significant ($\alpha = 0.05$) differences in mean percent hatch or mean percent survival of fathead minnow fry were seen at exposure levels up to 5.8 mg/L. However, the mean total length of 30-day-old fathead minnow fry continuously exposed to 5.8 mg/L was 10 percent less (statistically significant at $\alpha = 0.05$) than that of controls (Bentley et al. 1977).

Bentley et al. (1977) also studied the chronic toxic effects of RDX on fathead minnows. In the first study, which was accidently terminated at 140 days, mean RDX concentrations were 0.29, 0.64, 1.1, 2.7, and 4.9 mg/L. No significant effects ($\alpha = 0.05$) were observed on percentage of fry hatched or on fry survival and total lengths during the first 30 days of exposure. After 60 days of exposure, survival of fry at the highest concentration was reduced from controls, but total length was unaffected. From 63 to 140 days, no additional mortality or abnormal development was observed. Another similar test was initiated at this

time, with mean RDX concentrations of 0.43, 0.78, 1.5, 3.0, and 6.3 mg/L. A similar level of spawning activity was recorded in all treatment and control tests, with no significant differences ($\alpha = 0.05$) in total number of spawns, total number of eggs, or mean number of eggs per spawn. As in the previous test, percent hatch and total length were unaffected at all concentrations, but a significant ($\alpha = 0.05$) increase in fry mortality at 6.3 mg/L was seen after 30 days, with a further increase at 60 days. From days 64 to 240, survival, total length, and wet weight of mature fathead minnows remained unaffected at all exposure levels (Bentley et al. 1977).

3.3 TOXICITY TO MICROORGANISMS AND PLANTS

3.3.1 Bacteria

No studies were found specifically investigating the toxic effects of RDX on bacteria, either in cultures or in field situations. However, RDX can be biodegraded by the bacteria Pseudomonas spp., Alcaligenes spp., Thiorhodaceae (photosynthetic), Athiorhodaceae (photosynthetic), and Corynebacterium (Green 1972, as reported in Sullivan et al. 1979, Soli 1973, and Yang 1983), indicating that at the concentrations studied (from 0.05 up to 30 ppm), RDX may be considered nontoxic to these organisms. In addition, mutagenicity studies using the Ames Salmonella assay reported negative results for RDX at concentrations up to 2.5 mg/plate (Simmon et al. 1977, Stilwell et al. 1977, and Whong et al. 1980), also indicating that RDX is not toxic to these organisms.

3.3.2 Aquatic Algae

Bentley et al. (1977) studied the aquatic toxicity of RDX to two species of blue-green algae, Microcystis aeruginosa and Anabaena flos-aquae, the green algae Selenastrum capricornutum, and a species of diatom, Navicula pelliculosa. They exposed the four species for 96 hr in a static test to nominal concentrations of RDX of 0.0 (controls), 0.32, 1.0, 3.2, 10, and 32 mg/L, and evaluated changes in numbers of cells and in chlorophyll a content. All species showed decreases in cell density at 10 and 32 mg/L concentrations. RDX had a more pronounced effect on S. capricornutum at the high dose, with a decrease of 38 percent in cell density as compared to controls, and decreases from 2 to 23 percent at the other dose levels. Chlorophyll a content was decreased in all species but A. flos-aquae at concentrations of 1.0 mg/L and higher, with S. capricornutum affected at all concentrations. The decreases ranged from 1 to 26 percent and were greater at higher concentrations. S. capricornutum showed a decrease in chlorophyll a content of 16 to 17 percent at the 3.2 mg/L and 10 mg/L concentrations, and a decrease of 26 percent and 22 percent at the 10 mg/L and 32 mg/L concentrations, respectively. In general, M. aeruginosa, A. flos-aquae, and N. pelliculosa showed minimal (0 to 3 percent) decreases in cell growth and chlorophyll a content at all dose levels below 10 mg/L, and only 4 to 11 percent decreases at the 10 mg/L concentration.

Bentley et al. (1977) reported 96-hr EC_{50} values greater than 32 mg/L for all the algal species studied, and, based on the use of probit analysis, found nonsignificant differences ($\alpha = 0.05$) in cell density and chlorophyll *a* content. In a review of RDX toxicity, Sullivan et al. (1979) applied additional statistical treatments to the data of Bentley et al. (1977), and concluded that RDX significantly ($P \leq 0.05$) inhibited the growth of all four algal species after 96-hr of exposure, with inhibition most significant for *S. capricornutum*, even at the lowest dose level. Based on their statistical analysis of the Bentley et al. (1977) data, no-significant-effect-levels for alterations in cell density in the four species were as follows: 10 mg/L for *M. aeruginosa*, 3.2 mg/L for *A. flos-aquae*, <0.32 mg/L for *S. capricornutum*, and 3.2 mg/L for *N. pelliculosa* (Sullivan et al. 1979).

Sullivan et al. (1979) found *S. capricornutum* to be the only species of the four tested by Bentley et al. (1977) to exhibit a significant ($P \leq 0.05$) reduction in chlorophyll *a* content at all dose levels. They report a slight, but significant ($P \leq 0.05$), response at a concentration of 0.32 mg/L RDX after 96-hr of exposure. The inhibition of growth and chlorophyll *a* content in *S. capricornutum* at the 0.32 mg/L concentration was quite low (2 to 3 percent); the statistical methods employed were extremely sensitive, and Sullivan et al. point out that in this case, a slight change which shows statistical significance may not prove to be biologically significant.

Sullivan et al. (1977) conducted an aquatic field survey at Holston AAP to evaluate the impact of munition waste discharges on freshwater biotic communities. They report effects on periphyton in water containing as little as 20 µg/L RDX. However, they caution that a direct causal relationship between RDX concentrations and toxic effects is not warranted because of an inability to quantify actual conditions at any collecting station, unquantified environmental effects of carbon and nitrogen compounds present in the water, and the possible synergistic effects of RDX with other substances in the munition waste.

In a field survey of the aquatic systems affected by the Milan AAP, Huff et al. (1975) were unable to attribute any significant effect on the phytoplankton and periphyton populations to the RDX concentrations in the water. The observation of significant effluent effects was deemed impossible due to the absence of abundant plankton populations, attributed to high sediment loading, scouring, and short flow-through times during heavy runoff conditions. No other field data on plankton were found for RDX.

3.4 BIOACCUMULATION

Spanggord et al. (1980) reported data on biosorption of RDX by viable and heat-killed cells of bacteria. No significant biosorption was noted. In addition, no transformation by the bacteria occurred.

Bentley et al. (1977) studied the bioaccumulation of RDX in bluegill sunfish, channel catfish, and fathead minnows during 28 days of

continuous exposure to ^{14}C-labelled RDX. Tissues were wet-weighed, air-dried, and combusted, with the resulting $^{14}CO_2$ trapped in a scintillation vial. Activity was measured in a spectrometer. Uptake and elimination data are presented in Table 10 for each species at the two test concentrations used (0.014 and 1.0 mg/L). Bioaccumulation of ^{14}C-labelled residues in the visceral and edible tissues of these species occurred and reached a steady state after 14 days of exposure. At the lower concentration, edible tissue residues were quite small, with bioconcentration factors (BCF) ranging from 1.4 to 6.4. Mean measured ^{14}C-residues in edible tissue at the 1.0 mg/L concentration were 100 times higher than the lower dose level, although the BCF appeared somewhat lower (1.2 to 4.7). In the nonedible visceral tissue at both dose levels, the BCF was higher in bluegill sunfish and fathead minnows than it was in channel catfish. Uptake in viscera was about 2 to 3 times that found in edible tissue. As can be seen in Table 10, elimination of all RDX from the tissues at the low dose exposure occurred after 14 days in the bluegill sunfish and catfish but did not occur totally in the minnow. At the higher dose, minnows and catfish eliminated 70 to 87 percent of the accumulated RDX, but virtually no elimination occurred from either muscle or viscera in the bluegill sunfish.

Bioconcentration studies (Liu et al. 1983) were performed using 4-day exposures to ^{14}C-labelled RDX. Bioconcentration factors were estimated using the average amount of radioactivity recovered (disintegrations/min/g) in tissue and water. The 4-day BCFs were reported for S. capricornutum (123), D. magna (1.6), Lumbricus variegatus (3.0), and bluegill sunfish (1.9 in muscle and 3.1 in viscera). The BCF calculated for bluegill sunfish muscle and viscera is somewhat lower than that reported by Bentley et al. (1977). A summary of available bioconcentration data is presented in Table 11. Since the study of Bentley et al. (1977) was beset with dilutor malfunctions, only data from the early days of the bioaccumulation study are considered reliable, and are presented in Table 11.

3.5 SUMMARY

It appears that freshwater fish are more susceptible than freshwater invertebrates to RDX toxicity, having a range of LC_{50} values from 4.1 to 6.0 mg/L in 96-hr static tests, and 6.6 to 13 mg/L in 96-hr flow-through tests. Life cycle tests with fathead minnows resulted in chronic values very similar to, but slightly lower than, the acute values reported. Although no definitive 24-, 48-, 72-, or 96-hr EC_{50} values (based on immobilization) are available for freshwater invertebrates, values >15 mg/L in flow-through tests and >100 mg/L in static tests were reported for four invertebrate species.

Adequate long-term or steady-state studies have not been completed; however, bioconcentration of RDX in freshwater fish appears to be minimal, with reported values for edible tissue ranging from 1.9 to 4.7 in bluegill sunfish, 1.7 to 1.9 in channel catfish, and 1.4 to 2.0 in fathead minnows (Table 11).

TABLE 10. MEASURED ^{14}C-RESIDUES[a] CALCULATED AS RDX[b] IN THE EDIBLE AND VISCERAL TISSUE OF BLUEGILL (Lepomis macrochirus), CHANNEL CATFISH (Ictalurus punctatus), AND FATHEAD MINNOW (Pimephales promelas)[c]

		Mean Measured ^{14}C-Residues (mg/kg)							
		0.014 mg/L Dose				1.0 mg/L Dose			
Period	Day	Edible Tissue	Xd	Viscera	Xd	Edible Tissue	Xd	Viscera	Xd
				Bluegill					
Exposure	1	0.029 (0.017)[e]	3.1	0.072 (0.059)	8	2.9 (1.3)	2.9	8.1 (4.1)	8
	3	0.035 (0.011)	3.5	0.095 (0.047)	9.5	4.3 (2.2)	4.3	13 (7)	13
	7	0.038 (0.016)	2.5[f]	0.074 (0.048)	5[f]	4.0 (1.8)	4.0	12 (5)	12
	10	0.048 (0.010)	1.9[f]	0.04 (0.011)	2[f]	4.7 (2.9)	4.7	12 (10)	12
	14	0.14 (0.01)	6.4	0.27 (0.04)	12	2.6 (1.5)	3.8[f]	7.8 (4.1)	11[f]
	21	0.048 (0.007)	4.8	0.13 (0.06)	10	3.0 (0.3)	4.1	7.5 (0.3)	10
	28	0.046 (0.005)	4.7	0.091 (0.014)	9	3.8 (0.1)	3.5	6.8 (1.1)	6
Depuration	1	0.040 (0.005)		0.067 (0.019		3.1 (1.4)		8.4 (4.8)	
	3	0.049 (0.022)		0.083 (0.023)		3.0 (0.8)		3.5 -	
	7	0.033 (0.007)		0.064 (0.002)		1.5 (0.4)		2.7 (0.6)	
	10	0.031 (0.010)		0.074 (0.007)		2.0 (0.5)		4.0 (3.8)	
	14	<0.001		<0.002		3.6 (1.5)		6.4 (3.6)	
				Channel Catfish					
Exposure	1	0.016 (0.002)	1.7	0.027 (0.006)	2.9	1.7 (0.1)	1.7	2.8 (0.3)	2.8
	3	0.019 (0.003)	1.9	0.028 (0.002)	2.8	1.7 (0.3)	1.7	2.9 (0.5)	2.9
	7	0.033 (0.004)	2.0[f]	0.045 (0.002)	3[f]	1.8 (0.1)	1.8	3.2 (0.6)	3.2
	10	0.071 (0.008)	2.8[f]	0.12 (0.02)	4.8[f]	2.0 (0.3)	2.0	3.2 (0.2)	3.2
	14	0.12 (0.01)	5.5	0.12 (0.11)	5.5	0.08 (0.2)	1.2[f]	1.6 (0.4)	2.3[f]
	21	0.032 (0.004)	3.2	0.060 (0.005)	6	2.5 (0.3)	3.4	4.1 (1.1)	5.5
	28	0.039 (0.003)	4.0	0.049 (0.008)	5	3.2 (0.1)	2.9	3.6 (0.2)	3.3
Depuration	1	0.020 (0.002)		0.030 (0.006)		0.96 (0.19)		1.8 (0.3)	
	3	0.017 (0.001)		0.024 (0.006)		0.78 (0.15)		1.0 (0.4)	
	7	0.017 (0.004)		0.026 (0.006)		0.65 (0.16)		0.78 (0.15)	
	10	0.013 (0.002)		0.015 (0.002)		0.66 (0.06)		0.74 (0.08)	
	14	<0.002		<0.002		0.56 (0.12)		0.47 (0.27)	
				Fathead Minnow					
Exposure	1	0.013 (0.003)	1.4	0.023 (0.002)	2.5	1.4 (0.2)	1.4	2.2 (0.2)	2.2
	3	0.018 (0.004)	1.8	0.033 (0.003)	3.3	2.0 (0.1)	2.0	3.8 (0.5)	3.8
	7	0.031 (0.008)	2.1[f]	0.047 (0.005)	3.2[f]	1.8 (0.2)	1.8	3.4 (0.1)	3.4
	10	0.074 (0.004)	3.0[f]	0.15 (0.01)	6[f]	2.1 (0.3)	2.1	4.1 (0.6)	4.1
	14	0.13 (0.01)	5.9	0.26 (0.03)	12	1.4 (0.5)	3.8[f]	3.2 (0.8)	4.6[f]
	21	0.047 (0.007)	4.7	0.047 (0.011)	10	3.2 (0.7)	4.2	7.7 (0.3)	10
	28	0.058 (0.012)	5.9	0.11 (0.02)	11	4.4 (0.4)	4.0	9.6 (0.7)	8.8
Depuration	1	0.037 (0.011)		0.070 (0.006)		2.2 (1.2)		4.5 (0.1)	
	3	0.023 (0.004)		0.071 (0.043)		2.1 (0.4)		3.8 (0.4)	
	7	0.028 (0.012)		0.050 (0.005)		1.8 (0.5)		3.4 (0.6)	
	10	0.029 (0.008)		0.038 (0.005)		2.2 (1.0)		3.2 (0.3)	
	14	0.029 (0.007)		0.032 (0.006)		1.3 (0.9)		1.6 (0.5)	

a. Residues resulted from 28 days of continuous exposure to ^{14}C-RDX concentrations of 0.014 and 1.0 mg/L and from a 14-day period of depuration.

b. RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine.

c. Adapted from Bentley et al. (1977).

d. Bioconcentration factor based on the mean concentration of ^{14}C-RDX in water of the test day and the previous day.

e. Mean and standard deviation.

f. Bentley et al. (1977) report diluter malfunctions causing higher than average water concentrations on days 7 and 10 during the 0.014 mg/L study and lower than average water concentrations on day 14 of the 1.0-mg/L study.

TABLE 11. BIOACCUMULATION OF RDX[a] BY FRESHWATER AQUATIC ORGANISMS

Test Species	Tissue	Test Duration (days)	Water Concentration (mg/L)	BCF	Reference
Lepomis macrochirus	Muscle	1	0.014	3.1	Bentley et al. (1977)
			1.0	2.9	Bentley et al. (1977)
		3	0.014	3.5	Bentley et al. (1977)
			1.0	4.3	Bentley et al. (1977)
		4	0.3	1.9	Liu et al. (1983)
		10	1.0	4.7	Bentley et al. (1977)
Ictalurus punctatus	Muscle	1	0.014	1.7	Bentley et al. (1977)
			1.0	1.7	Bentley et al. (1977)
		3	0.014	1.9	Bentley et al. (1977)
			1.0	1.7	Bentley et al. (1977)
		10	1.0	2.0	Bentley et al. (1977)
Pimephales promelas	Muscle	1	0.014	1.4	Bentley et al. (1977)
			1.0	1.4	Bentley et al. (1977)
		3	0.014	1.8	Bentley et al. (1977)
			1.0	2.0	Bentley et al. (1977)
		10	1.0	2.1	Bentley et al. (1977)
Daphnia magna	Intact	4	0.3	1.6	Liu et al. (1983)
Lumbriculus variegatus	Intact	4	0.3	3.0	Liu et al. (1983)
Selenastrum capricornutum	Intact	4	0.3	123	Liu et al. (1983)

a. RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine.

Insufficient data are available to determine the toxicity of RDX to freshwater plants. No studies have been performed with saltwater vertebrates, invertebrates, or plants.

4. MAMMALIAN TOXICOLOGY AND HUMAN HEALTH EFFECTS

4.1 PHARMACOKINETICS

In humans and laboratory animals, RDX is slowly absorbed from the stomach after ingestion and also apparently from the lungs after inhalation. In laboratory animals it appears to be extensively metabolized in the liver, does not accumulate appreciably in any tissue, and is excreted primarily in the urine or exhaled as carbon dioxide (CO_2).

4.1.1 Absorption

Studies by von Oettingen et al. (1949) appeared to indicate that RDX was only very slowly absorbed from the gastrointestinal (GI) tract of dogs administered 5 and 15 mg/kg RDX orally, and 5, 10, and 20 mg/kg intraintestinally, as no change in various physiological functions was apparent 3 hr post-administration. These results were questioned, however, by McNamara et al. (1974) who suggested that the pretreatment of the dogs with the barbituate amytal may have influenced the absorption of RDX from the GI tract, and may have been responsible for the apparent lack of physiological effects in the dogs. An incident of accidental ingestion of a plastic explosive containing RDX by a German shepherd dog was reported in the literature (Berry et al. 1983), and did not involve any treatment with barbituates until after the onset of toxic effects. Symptoms of central nervous system (CNS) involvement appeared 8.5 hr following ingestion of an unknown quantity of RDX, and an epileptiform seizure occurred an hour later. This report indicates that RDX may be slowly absorbed from the GI tract following ingestion, taking 8 to 10 hr to cause a toxic reaction.

Twenty-four hours following oral administration to rats of 50 mg/kg ^{14}C-RDX dissolved in DMSO, 37 to 39 percent of the total radioactivity was still present in the gut; less than 3 percent was present after 48 hr (Schneider et al. 1976 a,b). Schneider et al. (1977) found that rats convulsed within the first several hours following RDX administration by gavage, while miniature swine convulsed 12 to 24 hr later. These findings are consistent with the time course for the appearance of RDX in plasma for the two species, RDX plasma levels peaking within 1 hr in rats, and between 6 to 24 hr in miniature swine. This latent period between RDX intake and the appearance of toxic manifestations in swine is consistent with that reported for humans following accidental or intentional inhalation or ingestion of RDX (e.g., Barsotti and Crotti 1949; Kaplan et al. 1965; Stone et al. 1969; Knepshield and Stone 1972; Ketel and Hughes 1972), and indicate that RDX is slowly absorbed from the GI tract. Schneider et al. (1977) suggest that miniature swine may be more similar to humans than rats, and more appropriate for the study of RDX metabolism and toxicity.

4.1.2 Distribution

Schneider et al. (1977) studied the toxic effects and metabolism of RDX in adult Sprague-Dawley rats of both sexes. In all of their studies, RDX was administered either as a slurry in isotonic saline or dissolved in DMSO. Ten rats were dosed intraperitoneally (i.p.) with 500 mg/kg RDX; all had multiple clonic/tonic convulsions, and eight out of ten rats died in less than 6 hr. Mean interpolated plasma RDX concentration at first seizure was 5.2 ± 0.4 μg/mL and at death was 13.8 ± 2.6 μg/mL. Mean RDX concentration in tissues at death was found to be 2.1 to 4.8 times that in plasma, with the highest concentrations occurring in the kidney (56.8 ± 5.9 μg/g). Brain concentrations at death were 29.5 ± 2.7 μg/g. Two rats dosed i.p. with 50 mg/kg RDX were sacrificed after 2 hr. Once again, tissue concentrations were higher (2.6 to 8.8 times) than plasma concentrations (1.05 ± 0.05 μg/mL), with brain concentrations 3.7 ± 0.6 μg/g, and the highest concentration found in the kidneys (9.4 ± 0.4 μg/g).

In this same study, plasma and tissue RDX concentrations were determined for groups of ten rats that were fasted for 24 hr, dosed by gavage with 100 mg/kg of a coarse, granular preparation of RDX, and sacrificed within 24 hr (Table 12). It can be seen that concentrations of RDX in all tissue samples except the liver varied by only about a factor of 2 over the 24-hr period. None of these rats convulsed, an observation consistent with the lower plasma and tissue concentrations compared with those dosed i.p. with 500 mg/kg (see previous paragraph). Liver RDX concentrations varied greatly (0.15 ± 0.15 to 8.51 ± 2.24 μg/g), and showed peaks at 2, 12, and 24 hr, perhaps indicating diurnal variation in the hepatic metabolism of RDX. All ten rats dosed by gavage with 50 mg/kg, using a more finely powdered RDX than in the previous experiments, convulsed within 2 hr of dosing, and two of the rats died. Plasma levels of surviving rats rose steadily to about 5 μg/mL at the first day following administration, and then dropped to negligible levels at four days following dosing.

In summary, in the experiments of Schneider et al. (1977), plasma concentrations in rats reached a dose-dependent plateau within several hours, were maintained at these levels for 24 hr, and then declined over the next two days. When convulsions occurred in rats, they were coincident with elevated RDX levels in plasma, and the onset appeared within the first few hours following administration of RDX.

Following single oral doses (by gavage) to miniature swine, RDX was found in all tissues sampled, indicating an extensive pattern of RDX metabolism similar to that observed in rats (Schneider et al. 1977). However, in contrast to the results from single oral doses to rats, no single tissue had higher RDX concentrations than other tissues. In the studies with swine, following administration of 100 mg/kg by gavage, urinary and plasma RDX concentrations were approximately equal after 24 hr. Absorption appeared to be slower; plasma and urine concentrations increased steadily between 6 and 24 hr after administration, coincident with the onset of convulsions 12 to 24 hr after dosing.

TABLE 12. PLASMA, URINE, AND TISSUE RDX CONCENTRATIONS AT VARIOUS TIMES AFTER DOSING RATS WITH 100 mg/kg BY GAVAGE

Time (hr)	Plasma (μg/mL)[a]	Urine (μg/mL)	Brain (μg/g)	Heart (μg/g)	Liver (μg/g)	Kidney (μg/g)
2	1.50 ± 0.26	2.45 ± 0.30	10.36 ± 1.24	7.97 ± 1.11	4.34 ± 0.90	12.86 ± 1.40
4	2.09 ± 0.09	5.46 ± 0.96	7.71 ± 0.98	6.49 ± 0.96	2.16 ± 0.56	12.30 ± 1.82
6	1.78 ± 0.15	5.02 ± 0.81	7.51 ± 0.57	7.13 ± 0.71	0.51 ± 0.34	13.58 ± 1.37
8	2.36 ± 0.22	7.31 ± 0.85	5.57 ± 0.67	3.82 ± 0.52	0.15 ± 0.15	10.90 ± 0.86
12	2.26 ± 0.16	5.49 ± 1.03	11.28 ± 1.60	11.08 ± 1.82	8.51 ± 2.24	22.02 ± 2.06
18	2.03 ± 0.10	5.58 ± 0.28	6.30 ± 0.20	5.58 ± 2.24	0.48 ± 0.20	12.12 ± 0.83
24	3.04 ± 0.48	6.87 ± 0.84	8.91 ± 1.07	7.89 ± 0.83	2.56 ± 1.15	16.85 ± 0.80

a. Conversion factor (μg/mL to μg/g) = 0.9737.

Source: Reprinted from Toxicology and Applied Pharmacology 39:531-541 (1977), by Schneider et al., with permission of Academic Press (London).

In order to study the distribution and elimination of RDX in plasma, ten rats were dosed intravenously (i.v.) with 5 to 6 mg/kg RDX dissolved in DMSO (Schneider et al. 1977). Plasma concentrations at 30 sec were 4.4 ± 0.35 µg/mL, and onset of convulsions occurred within seconds of administration, ceasing within 1 min following administration. After 6 hr, plasma RDX concentration had decreased to 1.9 ± 0.17 µg/mL, the disappearance being biphasic. The distribution phase half-time in plasma was 6.32 ± 0.18 min, the elimination phase was 10.1 ± 0.32 hr, and the volume of distribution was 2.18 L/kg.

Schneider et al. (1978) studied the distribution and metabolism of RDX in rats following subchronic exposure to RDX in food or drinking water. Sprague-Dawley rats of both sexes were either dosed by gavage with unlabelled RDX or ^{14}C-RDX (dissolved in DMSO) at 20 mg/kg/day for up to 90 days, or allowed free access to drinking water saturated with unlabelled RDX or ^{14}C-RDX (about 5 to 8 mg RDX/kg/day) for 90 days. Following ingestion of RDX-saturated drinking water, RDX did not accumulate excessively in any of the tissues examined. No overt signs of toxicity were observed during the study, and all organs appeared normal at necropsy. Mean plasma concentrations of RDX never exceeded 1.16 ± 0.06 µg/mL, and mean tissue levels ranged from 0.09 ± 0.04 to 0.84 ± 0.67 µg/g. Concentrations of RDX varied considerably in the liver, and to a lesser extent in other tissues at 30, 60, and 90 days.

Oral administration of RDX to rats in the 90-day studies of Schneider et al. (1978) resulted in elevated urine/plasma and tissue/plasma ratios, but no overall increase in tissue or plasma concentrations over time. Mean plasma levels of RDX in rats dosed by gavage with 20 mg/kg/day never exceeded 2.08 µg/mL, and mean tissue levels during the study ranged approximately from 5 to 10 µg/g. The 90-day fat RDX concentration was found to be elevated to about 20 µg/g, possibly a result of incomplete metabolism and excretion of RDX due to saturated metabolic pathways (Schneider et al. 1978). No convulsions or overt neurological signs of toxicity appeared in any of the treated rats; however, 8 out of 30 rats died during the course of this experiment, apparently from exacerbation of underlying chronic respiratory disease, as evidenced by necropsy findings (Schneider et al. 1978).

The time-course of RDX concentrations in rat blood and brain following per os (through the mouth) dosing with 50 mg/kg RDX is reported by MacPhail (1985). Finely powdered RDX was suspended in 2 percent carboxymethyl cellulose and administered per os to adult male Sprague-Dawley rats. Appreciable concentrations of RDX were detected in the blood at 2 hr after dosing (2.85 ± 0.38 µg/mL), with a gradual upward trend over time, reaching 4.0 ± 0.44 µg/mL at 24 hr. Levels dropped to 2.10 ± 0.37 µg/mL at 48 hr, and to 0.25 ± 0.03 µg/mL after 72 hr. RDX concentrations in the brain were 7.49 ± 1.01 µg/g at 2 hr, rose to 9.08 ± 0.90 µg/g at 24 hr, and dropped to 4.73 ± 1.26 µg/mL after 48 hr. After 72 hr, levels of RDX in the brain were not measurable. These values are very similar to those reported by Schneider et al. (1977), although the rats treated by gavage with 50 mg/kg finely powdered RDX in the Schneider et al. experiment all convulsed within 2 hr of treatment and two died (mean plasma RDX was 4.7 µg/mL). About 20 percent of the

rats dosed by gavage with 50 mg/kg labelled RDX in DMSO solution also died. The rats treated by MacPhail (1985) did not experience convulsions, and no deaths resulted.

4.1.3 Metabolism

Twenty-four hours after the oral administration of 50 mg/kg of 14C-RDX in DMSO to rats, about 39 percent of the total radioactivity was still present in the gut, but less than 3 percent was present after 48 hr (Schneider et al. 1977). Four days after administration, less than 10 percent of the original radioactivity remained in all tissues of the rat. Six days following administration of 50 mg/kg unlabeled RDX, about 2.4 percent of the RDX administered was excreted unchanged in the urine, 0.7 percent was found in the feces, and 0.6 percent remained in the carcass. However, during the four days following administration of 50 mg/kg labelled RDX, about 80 percent of the label was recovered from the urine or was exhaled as CO_2, about 4 percent was recovered in the feces, and 10 percent remained in the residual carcass (Schneider et al. 1977). These results indicate that RDX in the rat is extensively metabolized following ingestion.

After oral administration, greater amounts of radioactivity were found in the liver than were accounted for by its RDX content, indicating the presence of RDX metabolites (Schneider et al. 1977). A single oral dose to rats caused extensive long-lasting proliferation of the smooth endoplasmic reticulum in the liver, indicating the possible induction of the mixed function oxidase (MFO) system (French et al. 1976). Bradley (1977) also suggests that the metabolism of RDX is associated with the MFO system, because phenobarbital, an MFO inducer, increased the rate of RDX metabolism while pyrazole, an MFO inhibitor, decreased the RDX metabolic rate. Results from studies in which RDX was administered to rats in various treatment regimes indicate that RDX administered chronically to rats may inhibit its own metabolism, as all regimens tested failed to induce the MFO (Bradley 1977). In order to determine whether RDX stimulates the hepatic microsomal enzyme system, Dilley et al. (1978) fed rats a diet containing 1 percent RDX for 3 weeks, and tested liver homogeneate for enzyme induction using three metabolic pathways. In rat liver microsomal enzyme assays, RDX acted to a limited extent as a microsomal enzyme inducer as evidenced by the stimulation of the metabolism of o-nitroanisole (O-demethylation). RDX showed no stimulatory activity in the metabolism of aminopyrene (N-demethylation) or aniline (aromatic hydroxylation) (Dilley et al. 1978).

Based on the above observations, it is generally concluded that the reactions involved in RDX metabolism are catalyzed by microsomal enzyme systems and occur primarily in the liver.

Metabolism of RDX produces several kinds of one-carbon fragments: CO_2 (Schneider et al. 1977), bicarbonate ion, and formic acid (Schneider et al. 1978, reporting unpublished observations of Andersen and Bradley). No larger intermediates have been identified.

4.1.4 Excretion

Sunderman (1944) investigated the fate of ingested RDX following administration of a single dose to two rats. Finely powdered RDX was mixed with powdered rat biscuit and water to form a thick mush administered per os. The amount of RDX excreted in the urine was only 1 to 2 percent of the total ingested (total amount not reported), while the RDX recovered in the feces of the two rats represented 40 and 90 percent of the total, respectively, evident as long as 21 days following ingestion. Only traces of nitrite were found in the feces.

In order to study excretion of RDX, a group of ten rats was dosed by gavage with 50 mg/kg of RDX in a finely powdered form in an isotonic saline (Schneider et al. 1977). All convulsed within 2 hr of dosing, and two died. Plasma and urine concentrations peaked 1 day following administration (about 4.8 and 9.8 µg/mL, respectively), and declined sharply until the 4th day, remaining approximately the same thereafter (about 0.5 µg/mL). Contrary to the findings of Sunderman (1944), total urinary and fecal excretion of RDX during the first six days was about 2.4 and 0.7 percent, respectively, of the total administered.

Excretion of ^{14}C-labelled RDX was measured after 1, 4, 8, and 13 weeks in urine, feces, and exhaled air during 90-day administration of RDX (5 to 8 mg/kg/day) in drinking water (Schneider et al. 1978). Recovery of the label in excreta for the four sampling periods ranged as follows: 22 to 35 percent in urine; 4 to 5 percent in feces; 27 to 51 percent as exhaled CO_2. The amount of parent compound measured in the urine accounted for only 3 to 5 percent of total urinary activity. Recovery of label one week after administration of 20 mg/kg/day ^{14}C-RDX in food was about 31 percent in exhaled CO_2 and 34 percent in urine (Schneider et al. 1978). These results further indicate that RDX is extensively metabolized following ingestion, with elimination of metabolites in the urine or exhalation of CO_2 from the lungs.

4.2 ACUTE TOXICITY

4.2.1 Human Studies

While no controlled human studies exist concerning the toxic effects of acute exposures to RDX, instances of accidental inhalation or ingestion of RDX by humans have been documented in the literature (Ketel and Hughes 1972; Hollander and Colbach 1969; Stone et al. 1969; Woody et al. 1986). RDX exerts its primary toxic effect on the central nervous system, but also involves the gastrointestinal and renal systems (Rosenblatt 1980). No clinical descriptions of fatality from exposure to RDX have been reported in the literature, although several fatal cases have been mentioned by various authors (Sunderman 1944; Vogel 1952, as reported in Kaplan et al. 1965, Tsa and Lee 1982, as reported in Woody et al. 1986). Sklyanskaya and Pozhariskii (1944) state that a number of cases of RDX poisoning, some fatal, appear in occupational records, although the original reference is not available, and there is no positive association with RDX.

Toxic symptoms have been observed in armed forces personnel following the intentional or accidental ingestion of composition C-4, a plastic explosive containing 91 percent RDX, 2.1 percent polyisobutylene, 1.6 percent motor oil, and 5.3 percent di-(2 ethylhexyl) sebacate (Stone et al. 1969). Composition C-4 may be used as a field cooking fuel when other sources of heat are unavailable. Careless use of it for this purpose may result in accidental ingestion or prolonged exposure to its fumes while it burns; ingestion of C-4 was implicated in many cases of generalized seizures reported in U.S. Army hospitals throughout the Republic of South Vietnam (Ketel and Hughes 1972; Stone et al. 1969; Hollander and Colbach 1969).

After ingestion or inhalation of C-4, acute toxic effects are seen within a few hours. Ketel and Hughes (1972) report that 40 cases of C-4 intoxication were treated at one hospital during a 1-yr period in Vietnam. Affected individuals exhibited signs of CNS, renal, and gastrointestinal toxicity. Symptomatic effects included hyperirritability, nausea, vomiting, generalized epileptiform seizures, and prolonged postictal confusion and amnesia. These effects appeared to be completely reversible. Complete clinical data for 18 of the 40 cases were available, and were summarized by Ketel and Hughes as follows. The signs and symptoms of C-4 toxicity usually began 8 to 12 hr following exposure, with seizures occurring only during the initial 24 to 36 hr of illness in 84 percent of the patients. The remaining patients (three) experienced seizures during a 48- to 60-hr period. Recovery was complete within one week for 72 percent of the patients. Signs of renal toxicity were very mild and occurred in only 16 percent of the individuals; gastrointestinal toxicity appeared in 88 percent of the patients, and involved nausea and frequent vomiting. Elevated levels of white blood cells were noted in 72 percent of the patients, although elevated levels of serum glutamic oxalacetic transaminase (SGOT) and serum bilirubin were not found. No fatalities were reported. The exact component of C-4 that causes toxic encephalopathy with seizures was not identified.

Stone et al. (1969) reported symptoms in six cases which required hospitalization in Vietnam as follows. Ingestion of C-4 was followed within several hours by generalized seizures, severe nausea and vomiting, muscle twitching, mentation changes, and hematuria. Seizures occurred in all patients, including two who had ingested about 25 g of plastic explosive, and one who had ingested 180 g. Anemia and loss of memory for recent events persisted for longer than one month (time of discharge) in the patient who had ingested the largest dose (180 g). No fatalities were reported. Abnormal laboratory findings included neutrophilic leukocytosis, elevated SGOT, elevated blood urea nitrogen, proteinuria, and hematuria. All abnormal values returned to normal within two weeks. The authors concluded that hepatic involvement was effectively excluded as there were no abnormal findings of liver function studies, and liver biopsies appeared normal. Stone et al. (1969) presumed that RDX was the sole cause of the toxic effects of C-4, as "the other components are large molecules in low concentrations, and are probably nontoxic in the quantities ingested."

Pach et al. (1980) describe a case in Poland where a 20-yr-old male ingested 1 cm^3 trotyl (TNT and RDX) and 1 cm^3 plastic explosive (RDX). Nine hours following ingestion, the man experienced nausea, stomach pain, and vomiting; 12.5 hr after ingestion, the man experienced convulsions and loss of consciousness. After 17.5 hr, the man was admitted to the toxicological clinic, and was observed for 45 days. Pach et al. (1980) report gastrointestinal ulcerations, damage of the liver, and typical post-ingestion symptoms. Headaches, nausea, and insomnia persisted after seven months following the episode. It is impossible to relate the toxicological symptoms observed in this individual to RDX alone, as the patient had ingested both TNT and RDX.

Weigel (1955) reported that 150 g pure RDX with 5 percent hexamethylene tetramine dinitrate (reportedly added to increase RDX toxicity) caused vomiting in a man, with no further damaging consequences. It is not clear in the report whether the intake was accidental or intentional, nor is the size or age of the man given. The absence of any symptoms of CNS toxicity at this apparently large dose level, and the lack of available information describing the patient, preclude the use of this data in establishing acute toxicity levels in humans.

During World War II, the Bachmann process, which requires an essentially closed system for the reaction mixture was used to manufacture RDX. Standard procedures did not involve the handling of dry RDX. However, primary irritation and sensitization dermatitis, particularly of the face and eyelids, occurred in workers exposed to reaction fumes released during the nitration process. In order to identify the substance that was causing the skin irritation during chronic, occupational exposures, patch-test studies with 95 volunteers were initiated (Sunderman 1944). The results of these short-term, acute studies indicated that all the solid components involved in the RDX reaction mixture (hexamine, RDX, HMX, etc.) produced a mild primary skin irritation after five days. Minimal erythematous lesions appeared in 14 percent of the volunteers treated with dry, powdered RDX; however, ten days following the initial exposure a second, two-day application of RDX resulted in no skin reaction in any of the volunteers. Experimental animals and human volunteers exposed to the fumes and distillate of the RDX reaction mixture also experienced various degrees of primary skin irritation. Repeated exposures failed to produce any sensitivity reaction. In a further experiment, six subjects previously exposed to fumes from the Bachman reaction were tested with condensate from the reaction mixture. Two of these subjects were extremely sensitive to the reaction fumes, two were moderately sensitive, and two had experienced no sensitivity. The first two subjects showed a severe skin reaction from the fume condensate, the second two showed moderate reactions, and the latter two showed almost no reaction. Patch testing using the individual compounds present in the fumes emitted during the Bachman process failed to produce any local skin lesions similar in intensity to those observed during the manufacture of RDX. In conclusion, Sunderman (1944) was unable to identify the component of the fumes which was responsible for the skin lesions observed among operators at the plant. Although probably inconclusive due to the small sample size, von Oettingen et al. (1949)

also reported no signs of irritation in a 48-hr patch test using dry RDX on one human subject.

Only one clinical report describing the pharmacokinetics of RDX in humans appears in the literature. Woody et al. (1986) report the incidence of generalized tonic-clonic seizures in a 14.5-kg, 3-yr-old, child. The child's mother worked in a plant manufacturing plastic explosives (Composition C-4, which contains 91 percent RDX), and concluded the child had ingested clumps of the plasticized explosive which adhered to her boots and clothing. The child was admitted to the hospital 4 hr after the onset of convulsions. Following admission, samples of blood, urine, stool, and cerebrospinal fluid (CSF) were collected. The child's seizures were controlled with pharmacological paralysis after unsuccessful administration of repeated iv doses of diazepam, phenytoin, and phenobarbitoal (30 mg/kg). Concentrations of RDX in urine and stool increased during the observation period, with peak concentrations appearing in urine (38.41 mg/L) at 48 hr and in stool (4,486 µg/g) at 96 hr following ingestion. The concentration in serum was 10.74 mg/L at 24 hr, dropping to 3.56 mg/L at 48 hr, 0.66 mg/L at 96 hr, and negligible levels at 120 hr. The concentration of RDX in CSF was 8.94 mg/L at 24 hr, and the CSF:serum ratio was 0.832. Based on the assumption that RDX plasma levels peaked 2 hr following ingestion, the calculated values for the apparent terminal elimination rate constant and the half-life were estimated to be 0.046 L/hr and 15.06 hr, respectively. Using the volume of distribution (2.2 L/kg) reported by Schneider et al. (1977) for rats, Woody et al. estimated that the child had ingested 84.82 mg/kg, or a total of 1.23 g. This estimate was based on the assumption that RDX was completely liberated from the C-4 plasticized matrix and absorbed within 2 hr of ingestion, and that there was no presystemic clearance prior to entry into the vascular system.

Woody et al. (1986) report that the peak concentration of RDX in the feces at 96 hr relative to the urine 48 hr after ingestion suggests that RDX was concentrated and more slowly excreted in the feces than in the urine. Although Sunderman (1944) reported similar results in two rats following acute ingestion of RDX, these results are contradictory to the studies of Schneider et al. (1977), in which the total urinary excretion of RDX was 3.4 times higher than the total fecal excretion of RDX 6 days post-dose. In the Sunderman study, 1 to 2 percent of ingested RDX was excreted in the urine, and 40 to 90 percent appeared in the feces. Schneider et al. only recovered 2.4 and 0.7 percent of the total RDX administered in urine and feces, respectively. Schneider et al. (1977) administered the RDX by gavage in a finely powdered slurry, which probably made the RDX more available for absorption and metabolism over that experienced following the ingestion of the RDX in the plasticized form of Composition C-4. Sunderman (1944) administered RDX in a finely powdered form mixed in rat biscuit, which might have produced an intermediate degree of absorption between the two others described above.

4.2.2 Animal Studies

As in man, CNS excitation is the most prominent acute effect of RDX on most animals. Other toxic manifestations include gasping and labored

breathing. Congestion in the kidney, liver, and lungs is often seen in necropsied animals following convulsions and death.

Table 13 lists representative lethality values reported in the literature for RDX. As indicated by the values in the table, RDX is moderately to highly toxic when administered to laboratory animals. As would be expected, the intravenous LD_{50} of RDX is much lower than the oral LD_{50}. There may be sex differences in response to RDX treatment (Levine et al. 1981; Cholakis et al. 1980; Dilley et al. 1978).

Oral LD_{50} values for the same species may differ among laboratories because the acute LD_{50} is dependent on the physical form of the RDX and on the method used to suspend or dissolve it (Schneider et al. 1977). Coarse, granulated powder produced an oral LD_{50} of 300 mg/kg in rats; finely powdered RDX in saline slurry produced the same LD_{50} value as when dissolved in DMSO, approximately 100 mg/kg. Differences in toxicities of the preparations were reflected in plasma RDX concentrations reached in rats dosed with different particle size RDX. The 24-hr plasma RDX value following administration of 50 mg/kg of the finely powdered RDX was 4.7 µg/mL, while it was only 3.04 µg/mL following a dose of 100 mg/kg in the coarse, granulated form (Schneider et al. 1977).

Although using a limited number of experimental animals, Sunderman (1944) found that there was a difference in response to acute RDX toxicity in fasting and nonfasting rats, as well as a difference in plasma RDX levels. In fasting rats, 1 death out of 2 animals occurred 20 hr following single oral doses of 50 and 100 mg/kg, with plasma concentrations 10.7 and 7.3 µg/mL, respectively. A minimum lethal dose (LD_{LO}) for fasted rats of 50 mg/kg is reported. In nonfasting rats, three deaths out of four rats occurred at 200 mg/kg, with only one recorded plasma RDX concentration, 3.0 µg/mL. In the latter, nonfasting, study the only rat treated at 75 mg/kg died (plasma RDX reported as 0.0 µg/mL), but four rats out of four survived at dose levels of 100 and 150 mg/kg, indicating a higher tolerance for RDX in nonfasted rats. Sunderman reports an LD_{LO} for nonfasted rats of 75 mg/kg. Dogs fed 25, 100, and 300 mg/kg RDX in meat exhibited no toxic effects at the lowest dose level, and only emesis at the higher concentrations (Sunderman 1944).

Sunderman (1944) also attempted to determine the relationship between route of administration and toxic effects of RDX. Intraperitoneal administration of a finely powdered suspension of RDX in saline solution produced 9 deaths out of 10 in nonfasted rats at a dose of 100 mg/kg (nonlethal when administered orally). Plasma RDX concentrations ranged from 2.0 to 16.6 µg/mL. A dose as low as 10 mg/kg i.p. produced convulsions (plasma RDX was reported as about 0 µg/mL), and 25 mg/kg i.p. resulted in convulsions and death (plasma RDX 6.7 µg/mL). Subcutaneous injections of 100 mg/kg resulted in 100 percent convulsions, and two deaths out of three rats. Intravenous injection of 18 mg/kg in a rabbit was followed by severe convulsions and death. This may be compared to the oral lethal dose to rabbits of 500 mg/kg reported by Kaplan et al. (1965, reporting data of Sklyanskaya and Pozhariskii 1944). The results of Sunderman (1944) indicate that the toxic effects following

TABLE 13. RDX LETHALITY DATA

Species	Route/Vehicle	LD_{50}[a] (mg/kg)	Reference
Rat	Oral/saffron oil	44	Lehman et al. 1965
	Oral/corn oil	70 (immature male) 50-75 (immature female)	Dilley et al. 1978
	Oral/food	50 (LD_{LO}[b] - fasted)	Sunderman 1944
	Oral/food	75 (LD_{LO} - nonfasted)	Sunderman 1944
	Oral/DMSO or saline slurry	100	Schneider et al. 1977
	Oral/methyl cellulose-polysorbate 80	118 (combined sexes)	Cholakis et al. 1980
	Oral/food	153	Kaczorowski and Syrowatka 1960 as reported in Chem. Abstr.
	Oral/gum acacia	200	von Oettingen et al. 1949
	Oral/coarse powder	300	Schneider et al. 1977
	Intraperitoneal/saline solution	25 (LD_{LO})	Sunderman 1944
Mouse	Oral/aqueous solution	500	Sklyanskaya and Pozhariskii 1944, as reported in Kaplan et al. 1965
	Oral/corn oil	<75 (immature male) 86 (immature female)	Dilley et al. 1978a
	Oral/methyl cellulose-polysorbate 80	80 (combined sexes) 97.2 (male) 58.9 (female)	Cholakis et al. 1980
	Intravenous/DMSO	19	McNamara et al. 1974
Cat	Oral/linseed oil	100 (LD_{LO})	Sklyanskaya and Pozhariskii 1944, as reported in Kaplan et al. 1965
Rabbit	Oral/linseed oil	500 (LD_{LO})	Sklyanskaya and Pozhariskii 1944, as reported in Kaplan et al. 1965
	Intravenous/saline solution	18 (LD_{LO})	Sunderman 1944
Guinea pig	Intravenous/DMSO	25	McNamara et al. 1974
Dog	Intravenous/DMSO	40 (LD_{100})[c]	McNamara et al. 1974

a. Except as otherwise noted.

b. LD_{LO} = the lowest dose at which mortality was observed.

c. LD_{100} = the dose at which 100% mortality occurred.

parenteral administration of RDX are similar to those observed following oral administration in rats, but show that the onset of effects is more rapid, and the lowest-observed-adverse-effect-level is lower with parenteral administration. Pathologic findings in rats were similar no matter what the method of dosing, and included congestion of the liver, kidneys, and lungs, with liver and kidney degeneration and necrosis. Sunderman concluded that his experiments support the view that the toxicity of RDX is probably not the result of changes in its chemical composition due to breakdown by GI secretions, but is the direct result of absorbed RDX.

In order to determine the acute toxicity of RDX to rats, von Oettingen et al. (1949) administered single doses of RDX suspended in gum acacia solution via stomach intubation. Doses ranging from 25 to 400 mg/kg (in 1, 2, and 4 percent suspensions) resulted in 0 to 100 percent mortality, with deaths occurring in all but the lowest dose group. The majority of deaths occurred within 24 hr. At necropsy there was moderate to marked congestion of the GI tract and lungs in some animals. An oral LD_{50} of 200 mg/kg for RDX administered in a 4 percent suspension to rats was estimated.

The acute effects of RDX on the physiological functions of dogs were also studied by von Oettingen et al. (1949). Oral administration of 5 and 15 mg/kg RDX suspended in a 10 percent gum acacia solution was found to cause no distinctive changes in spinal pressure, respiration, or circulation over a 4-hr period. Doses of 5, 10, and 20 mg/kg were injected into the duodenum or jejunum in an attempt to increase absorption, but these experiments also failed to show any change in the physiologic functions studied.

Lehmann et al. (1979) administered acute oral doses of RDX suspended in saffron oil to fasting rats. Doses ranged from 5 to 300 mg/kg, and the authors estimated an LD_{50} of 44 mg/kg with a 95 percent confidence limit of 30 to 64 mg/kg. The animals died approximately 30 min following administration of RDX, demonstrating ataxia, intense salivation, and clonic/tonic convulsions.

Acute oral lethality studies in Fischer 344 rats and B6C3F1 mice were performed by Cholakis et al. (1980). Animals were fasted overnight and given gavage doses of RDX suspended in 1 percent methylcellulose in water (rats) or 1 percent methylcellulose and 1 percent polysorbate 80 (mice). The RDX used in the study was contaminated with 9 percent HMX. In the rat study, using ten rats of each sex, all of the animals died within 2 to 3 hr after RDX administration at doses of 150, 180, and 250 mg/kg. At a dose level of 200 mg/kg, 95 percent of the animals died in the same time period. Mortality was 70 percent and 5 percent at the doses of 125 and 100 mg/kg, respectively. There were no apparent sex differences found in response to RDX treatment. An acute oral LD_{50} of 118.1 mg/kg for the combined sexes was estimated.

Five mice of each sex were fasted overnight and treated with 60, 100, 140, 180, 225, or 350 mg/kg, with 90 to 100 percent mortality occurring at the four highest dose levels (Cholakis et al. 1980). Sixty

percent and 30 percent mortality occurred at the 100 and 60 mg/kg concentrations, respectively. Death occurred in all of the mice within 5 to 10 min following administration at the three highest dose levels; at the 100 and 140 mg/kg concentrations, death occurred within 30 min; at the lowest dose level, no toxic effects were observed, and death was delayed until day 10 of the test. The LD_{50} value for the combined sexes was 80.3 mg/kg. The authors suggested that there may be apparent sex differences in response of mice to RDX treatment but that overlapping confidence limits do not support that claim.

In both the rat and mouse studies, toxic effects preceding death included gasping, labored breathing, and clonic/tonic convulsions, indicating neurotoxicity. Cholakis et al. (1980) report that in the mice treated with RDX, convulsions could be induced by a finger snap at all but the lowest dose level, 60 mg/kg.

McNamara et al. (1974) studied the acute effects on rabbits of single cutaneous applications (24 hr) of RDX dissolved in DMSO, cyclohexanone, or acetone (doses of 165, 37.5, and 27 mg RDX/kg, respectively). Single doses of 1.0 mL RDX in the solvents produced no gross evidence of cutaneous irritation or systemic effects in rabbits throughout a 30-day observation period. No changes in blood constituents were noted. Microscopic examination of the dosed areas showed that RDX in all solvents resulted in dermatitis persisting for as long as 30 days, while solvent control animals experienced no dermatitis. Similar cutaneous studies with dogs using RDX dissolved in DMSO (289 mg/kg), cyclohexanone (47.3 mg/kg), or acetone (65.7 mg/kg) found no changes in blood pressure, respiratory and heart rates, EKG, or EEG during exposure or for four weeks following exposure.

MacPhail (1984, 1985) has completed a preliminary evaluation of the neurobehavioral toxicity of RDX exposure in rats, investigating the acute effects of RDX on schedule-controlled behavior, flavor-aversion conditioning, motor activity, and landing footspread. Details of the study are not completely described, and the information is in the form of progress reports to the U.S. Army Medical Bioengineering Research and Development Laboratory (USAMBRDL). The information given is as follows. Adult female rats were trained to respond (press a key) under a schedule of milk reinforcement, and once performance had stabilized, were treated with 50 mg/kg RDX per os, 2 hr presession. Response rate (in responses per second) under a variable-interval (VI) 90-sec schedule, and a variable-ratio (VR) 50-response schedule were measured. The results show that RDX reduced VR response by 87 percent and VI response by 63 percent from pretreatment levels. Recovery from the effect of RDX was found to be gradual, with baseline performance not reinstated until 72 hr after dosing (MacPhail 1984). MacPhail suggests that this slow recovery may be a reflection of the relatively slow elimination of RDX in plasma as shown by Schneider et al. (1977).

Acute dose-response functions were also obtained for performance under the VI and VR schedules (MacPhail 1984). Female rats were divided into groups of eight, and treated per os with 12.5, 25, or 50 mg/kg RDX or the vehicle (2 percent carboxymethyl cellulose). RDX was found to

produce substantial dose-related decreases in response under both schedules, with an ED_{50} value (the dose that results in a response that is 50 percent of the vehicle control value) of 26.8 mg/kg for the VI 90-sec schedule. In the VR 50-response schedule, even the smallest dose produced greater than a 50 percent reduction in response rate.

In another experiment, adult male rats (N = 9 per group) were adapted to limited water availability (30 min/day) and after intake had stabilized, were given access to saccharin and then dosed per os with RDX (12.5, 25, or 50 mg/kg) or the vehicle (MacPhail 1984). Pairing saccharin intake with RDX resulted in an almost complete aversion to saccharin at all dose levels one day after dosing, and a dose-related decrease in total water consumption (from about 17 mL at the lowest dose to about 6 mL at the 50 mg/kg concentration, compared to about 22 mL consumed by vehicle-treated rats). Three days after dosing, the rats were again given the choice between saccharin and tap water, and the choice testing revealed that the conditioned flavor aversion was intermediate in rats treated previously at the lowest dose level, and nearly complete in rats treated previously at the 25 and 50 mg/kg concentrations. At this time, total fluid intake had almost totally recovered.

General motor competence of rats was measured in a figure-eight maze following per os administration of 0 (vehicle control), 12.5, 25, or 50 mg/kg RDX (MacPhail 1985). RDX (given 2 hr presession) was found to produce a dose-related decrease in motor activity (measured in counts/session), with approximately 60 and 77 percent reductions in motor activity observed when compared to controls at the lowest and highest dose levels, respectively. Effects were still apparent when the rats were again tested 24 hr after dosing. RDX was also found to decrease landing footspread (measured as hindleg splay) in these same rats, but in a dose-independent fashion.

Burdette and Dyer (1985) studied audiogenic-induced seizures in rats treated per os with 0, 10, 20, or 60 mg/kg RDX. They found that 1 min stimulation with ultrasound 8 hr following administration of RDX resulted in seizures in 10 percent of the rats at 10 mg/kg, 30 percent at 20 mg/kg, and 82 percent at 60 mg/kg. The duration of pentylenetetrazol-induced (50 mg/kg i.p.) seizures was increased in rats given 60 mg/kg RDX.

4.3 SUBCHRONIC AND CHRONIC TOXICITY

4.3.1 Human Studies

In the munitions industry, RDX exposure has occurred mainly from inhalation of fine particles; because RDX is not very lipid soluble, skin absorption is very unlikely (Rosenblatt 1980). Chronic intoxication in workers is characterized by epileptiform seizures (generalized convulsions) and unconsciousness (Stokinger et al. 1982). Convulsions may appear without warning or be preceded by one or two days of insomnia, restlessness, and irritability. Seizures are followed by temporary amnesia, disorientation, and asthenia.

Kaplan et al. (1965) described five cases of convulsions and/or unconsciousness occurring among 26 workers employed in a U.S. explosives plant processing RDX. The plant had only operated from April to July, 1962, so the exposure period was limited to this time. The workers were exposed to RDX by the release of dust into the workroom air during dumping, screening, and blending of dried RDX powder, and cleanup of any spilled material. Inhalation or ingestion of the finely powdered dust was considered responsible for the toxic effects observed. The typical symptomatology either at work or several hours after work included sudden convulsions and/or unconsciousness. Following a period of unconsciousness lasting from several minutes to 24 hr, varying periods of stupor, disorientation, nausea, and weakness ensued. Recovery was apparently complete for all affected personnel. All of the men who became ill stated that they had failed to wear their respirators continuously and had ignored other personal hygiene directives (such as regular showers and washing of hands). Improved ventilation systems in the work areas and more strictly enforced use of personal protective measures resulted in the absence of any further cases of RDX intoxication.

Similar evidence of systemic intoxication was not observed at the Holston Ordnance Works during World War II, although Sunderman (1944) suggests that insufficient time had elapsed for evidence of chronic toxicity among workers.

An industrial hygiene study of occupational diseases in U.S. government-owned explosives plants during World War II was summarized by McConnell et al. (1946). Over 37 months' operating time, an average population of 36,400 was employed. During this time (112,000 man-years) there were 626 cases of mild dermatitis attributed to exposure to RDX (composition B), but no reported cases of systemic poisoning or fatalities. Composition B is composed of a 60/40 mixture of RDX and TNT, with 1 percent of synthetic wax added (Urbanski 1984). It is unclear in the McConnell et al. report whether the dermatitis was actually caused by RDX alone, or by the composition B mix.

In 1974 a cross-sectional epidemiologic study of 1491 volunteers working at five U.S. AAPs was conducted to investigate a possible association between RDX exposure and the relatively rare disease, systemic lupus erythematosis (SLE). This study resulted from a report of three cases of SLE in two years among RDX-exposed workers at one of the plants and the structural similarity of RDX to various drugs known to induce SLE (Hathaway and Buck 1977). Of the volunteers in the study, 58 percent were not exposed to explosives, 4 percent were exposed to TNT alone, 31 and 2 percent were exposed to RDX in combination with TNT and HMX, respectively, and 5 percent were exposed to RDX alone. Only employees exposed to RDX alone or RDX mixed with HMX were included in the study. The investigators attempted to identify abnormalities of the hematologic, hepatic, and renal systems, and the presence of autoimmune disease. Results of the study showed no additional cases of SLE or excess of autoimmune disease, and no statistically significant differences (level of significance not reported) in abnormalities of the hematologic, hepatic, or renal systems in employees with 8-hr time-weighted

exposures to RDX of up to 1.57 mg/m^3 (0.28 mg/m^3 average) compared to unexposed controls. The authors concluded that the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) for RDX of 1.5 mg/m^3 was a safe exposure level (Hathaway and Buck 1977).

Various other occupational cases of RDX toxicity have been reported in the European literature. Barsotti and Crotti (1949) reported 17 cases of convulsive fits, loss of consciousness, and disorientaion among 20 Italian workers exposed to inhalation of powdered RDX during its manufacture. The authors suggested that the epileptic fits and other symptomatic effects were due to cortical angiospastic crises caused by the absorption of RDX. Tobacco and alcohol were thought to be aggravating factors. No fatalities were reported, and recovery in all cases was complete (Barsotti and Crotti 1949; and as reported in Kaplan et al. 1965).

Vogel (1952) likewise reported toxic effects observed among German workers specifically involved in the handling of dry, finely pulverized RDX. Exposure was thought to occur from inhalation or ingestion of the particles, and resulted in epileptiform attacks, as well as the other pre- and postexposure symptoms described above. Severe irritation of the skin, oral mucous membranes, and conjunctiva was observed in a few susceptible persons.

Several poisonings were reported as having occurred in a Hungarian plant where trotyl (an explosive containing RDX) was handled (Valyi-Nagy et al. 1947, as reported in Chem. Abstr. 1949), although no description of symptoms was given. The authors found that the best protection was afforded by the use of gas masks filled with active carbon, pumice, and linseed oil-saturated chips.

4.3.2 Animal Studies

Subchronic and chronic toxicity studies, some of them directed toward determining a no-effect dose level and a water quality criterion, are summarized in Tables 14 and 15, and will be described below.

4.3.2.1 Subchronic Studies

In subchronic feeding studies, six rats were fed daily doses of 50 mg/kg RDX for a period of 5 to 78 days (Sunderman 1944). Four animals experienced convulsions, four died in less than ten days, and one died a week after the experiment was discontinued. Although blood counts and hemoglobin levels were normal, appreciable concentrations of RDX were measured in the blood even in cases where no toxic manifestations were observed. The concentration of RDX in the blood at death ranged from 2.7 to 8.3 µg/mL. Necropsies performed on all the animals that died either in the acute or subchronic toxicity studies showed congestion of the liver, lungs, kidneys, and intestinal tract. Areas of hyaline degeneration in heart muscle, tubular epithelial degeneration in kidneys, and fatty degeneration in the liver were found. No congestion in cerebral vessels was noted. Sunderman attributed the congestive effects

TABLE 14. SUMMARY OF RDX[a] SUBCHRONIC TOXICITY STUDIES

Species	Daily Dose[b]	Test Duration	Effects	Reference
Rat	50 mg/kg	5-78 days	Convulsions, and death in 5/6 animals: blood count, hemoglobin normal: congestion of liver, kidneys, lungs, and intestine: degeneration and necrosis in liver and kidney: hyaline degenration in heart muscle	Sunderman 1944
	25, 50, 100 mg/kg	10 weeks	Weight loss, hyperactivity, convulsions, 40-87% mortality: congestion of lungs and GI tract	von Oettingen et al. 1949
	15 mg/kg	10 weeks	No toxic symptoms or death	von Oettingen et al. 1949
	300, 600 mg/kg	13 weeks 3 weeks	Mortality in less than three weeks	Levine et al. 1981
	10, 30, 100 mg/kg	13 weeks	Hyperactivity to approach, convulsions: dose-related reductions in body weight gain: decreased serum triglycerides, marginal leukocytosis: mortality at 100 mg/kg/d	Levine et al. 1981
	0.3, 2.5, 6.5, 12.5 mg/kg	84 days	Decrease in growth rate at 6.5 and 12.5 mg/kg/day: dose-related, biphasic changes in brain MAO, cholinesterase, and oxygen uptake: neurotransmitter dynamics and behavior unchanged from controls: NOEL[c] of 0.3 mg/kg/day	Brown 1975
	40 mg/kg	90 days	Dose-related decreased weight gain and food consumption in males: no behavioral/pharmaco-toxic or clinical chemistry changes: decreased heart weights: dose-related hematological changes: myocardial degeneration, liver portal inflammation in females	Cholakis et al. 1980
	10, 14, 20, 28 mg/kg	90 days	Dose-related hematological changes at 28 mg/kg/day: NOEL of 14 mg/kg	Cholakis et al. 1980
	20 mg/kg	90 days	Lethargy, weight loss, rough coat hair: no neurological symptoms, death from exacerbation of chronic respiratory disease	Schneider et al. 1978
	5 to 8 mg/kg[d]	90 days	No signs of toxicity	Schneider et al. 1978
Mouse	10, 14, 20, 28, 40 mg/kg	90 days	No effects	Cholakis et al. 1980
	320 mg/kg	90 days	Hyperactivity, mortality: increased liver and kidney weight, hepatocellular vacuolization, tubular nephrosis	Cholakis et al. 1980
Dog	50 mg/kg	6 weeks	Weight loss, hyperactivity, hyperirritability, convulsions: no blood or tissue effects	von Oettingen et al. 1949
	0.1, 1.0, 10 mg/kg	90 days	Temporary episodes of emesis	Hart 1974
Rhesus monkey	0.1, 1, 10.0 mg/kg	90 days	CNS disturbance in 83% at 10 mg/kg/day: recovery complete: tonic convulsions and emesis only clinical signs of toxicity: hematology, urinalysis, blood chemistry normal: NOAEL[e] of 1.0 mg/kg/day	Litton Bionetics, Inc.

a. RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine.

b. All doses administered orally.

c. NOEL = no-observed-effect-level.

d. Administered in drinking water.

e. NOAEL = no-observed-adverse-effect-level.

TABLE 15. SUMMARY OF RDX[a] 2-yr CHRONIC TOXICITY STUDIES

Species	Daily Dose[b]	Effects	Reference
Rat	1.0, 3.1, 10 mg/kg	No evidence of toxicity	Hart 1976
	0.3, 1.5, 8.0, 40 mg/kg	Increase in mortality and decrease in mean survival time at high dose levels; no treatment-related lesions of CNS; toxic effects included anemia, hepatotoxicity, cataracts and urogenital lesions; LOAEL[c] of 1.5 mg/kg/day; NOEL[d] of 0.3 mg/kg/day reported	Levine et al. 1983
Mouse	100/175 mg/kg	Lethal for ~68%; high incidence of fighting wounds in males; reduction in body weight gain; hypercholesterolemia; hepatomegaly; increased kidney and heart weight; testicular degeneration	Levine et al. 1984
	35.0 mg/kg	Hypercholesterolemia in females; testicular degeneration; elevated renal weight in females at termination	Levine et al. 1984
	7.0 mg/kg	Possible hypercholesterolemia in females	Levine et al. 1984
	1.5 mg/kg	NOEL	Levine et al. 1984

a. RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine.

b. All doses administered orally.

c. LOAEL = lowest-observed-adverse-effect-level.

d. NOEL = no-observed-effect-level.

noted in organs to anoxemia resulting from spasms or perhaps paralysis of the respiratory muscles during RDX convulsions.

In an attempt to ascertain whether RDX was affecting the CNS, Sunderman (1944) found that administration of nembutal, an antispasmodic drug, prevented convulsions and subsequent death in six out of seven rats, and that decerebrated rats exhibited no convulsive symptoms after intraperitoneal injection of a dose of 100 mg/kg RDX (lethal in untreated rats).

In ten-week (70-day) studies with rats receiving 15 mg/kg daily with food, no abnormal symptoms or deaths were reported (von Oettingen et al. 1949). The ingestion of 50-100 mg/kg/day for ten weeks caused hyperirritability and clonic/tonic convulsions in rats, with 60 and 87 percent mortality in the 50 and 100 mg/kg groups, respectively. Another study by Von Oettingen et al. (1949) in which rats were fed 15, 25, or 50 mg/kg/day for 12 weeks resulted in 40 percent mortality at the 25 and 50 mg/kg/day dose levels. An early weight loss was regained by all animals by the end of the experiment. Necropsied animals in both the 10- and 12-week studies showed congestion of the lungs and GI tract. No abnormal systemic effects were noticed in the blood or tissues. In the 12-week study, one death in 20 rats was reported in the 15 mg/kg/day group, with necropsy revealing a large encapsulated cyst replacing one liver lobe.

In a further attempt to examine the toxic effects of repeated administration of RDX, von Oettingen et al. (1949) fed seven healthy dogs 50 mg/kg/day for 6 weeks. There was no evidence of toxic effects on the blood, blood-forming organs, heart, lungs, liver, spleen, brain, testes, adrenals, or kidneys. Weight loss was apparent, and CNS effects were demonstrated, including hyperirritability and convulsions. No deaths resulted at this dose level.

Dogs given daily oral doses of 0.1, 1, or 10 mg/kg RDX for 90 days developed no signs of toxicity except for temporary episodes of emesis. Laboratory diagnostic procedures as well as gross and microscopic postmortem examinations revealed no important differences from controls (Hart 1974).

Litton Bionetics, Inc. (1974), studied the subchronic toxicity of RDX in rhesus monkeys. Doses of 0, 0.1, 1, and 10 mg/kg/day RDX in an aqueous solution of methylcellulose were administered orally seven days a week for 13 weeks (90 days). Test and control groups consisted of three monkeys of each sex; due to the small sample size, the authors performed no statistical analysis of the results. Five monkeys in the high dose group (83 percent) exhibited signs of CNS disturbance on 12 occasions, usually involving tonic convulsions. Recovery was complete in all but one monkey. Plasma samples were obtained from three of the convulsing monkeys and plasma RDX concentrations were found to range from 2.0 to 3.7 mg/mL. Except for frequent episodes of vomiting, no other clinical signs of toxicity were observed in any of the dose groups. Laboratory tests (including hematology, urinalysis, and blood chemistry) showed only random changes and were judged to be of no

toxicological significance. Increases in numbers of degenerate or necrotic megakaryocytes in bone marrow sections were observed, as well as increased amounts of iron-positive material in liver cord cytoplasm, both occurring in the high dose group. The toxicological significance of these findings was not known. As a result of these studies, a no-observed effect-level (NOEL) of 1.0 mg/kg/day can be defined.

Brown (1975) studied the changes in brain biochemistry and behavior of rats exposed to daily administration of RDX in a range of four doses over a 12-week feeding period. The study was designed to establish the effect of RDX on oxygen uptake, determine its effect on brain cholinesterase and monoamine oxidase (MAO), and to establish the effect of RDX on neurotransmitter dynamics by investigating steady-state levels and turnover rates of brain norepinephrine, dopamine, and 5-hydroxytryptamine. Adult, male, Sprague-Dawley rats were administered (i.p.) 0, 0.3, 2.5, 6.5, 12.5, or 25 mg/kg/day RDX suspended in 1 percent carboxymethyl cellulose for a period of 12 weeks. Six rats were in each dose group, as well as six controls administered only the vehicle. Brown observed convulsions and 5 deaths out of 6 rats by day 8 at the highest dose level (25 mg/kg/day), but no overt signs of toxicity at the other dose levels studied, other than a 25 percent decrease in growth rate at the 6.5 and 12.5 mg/kg/day concentrations. Subchronic exposure to RDX was found to induce time- and dose-related, biphasic, changes in brain MAO, cholinesterase, and oxygen uptake. Blood levels of RDX were found to be dose-related. None of these effects were found in rats chronically dosed with 0.30 mg/kg/day for 12 weeks, thus Brown (1975) reported this as an NOEL. No remarkable changes were found in the neurotransmitter studies. Behavioral studies including seizure thresholds, spontaneous motor activity, and examination for gross neurological abnormalities found the dosed animals to be no different from controls. Brain weight, brain protein levels, and DNA-RNA were also unchanged from controls.

In 90-day subchronic toxicity studies, oral doses of 20 mg/kg/day RDX in food and about 5 to 8 mg/kg/day in RDX-saturated drinking water resulted in no convulsions or other signs of neurotoxicity in adult Sprague-Dawley rats; however, in the 20 mg/kg/day study, eight deaths out of thirty rats resulted from exacerbation of chronic respiratory disease (Schneider et al. 1978).

In another 90-day subchronic toxicity study, adult Fischer 344 rats and B6C3F1 hybrid mice were administered RDX (mixed with 9 percent HMX) in feed (Cholakis et al. 1980). Groups of ten males and ten females each received doses of 0 (controls), 10, 14, 20, 28, or 40 mg/kg/day. In the rat studies, 40 mg/kg/day was found to be toxic, while the 28 mg/kg/day dose was not. The only consistent toxic effect observed was decreased weight gain and decreased feed consumption in males during some of the weeks. No toxic effects were seen in mice at these dose levels.

In a follow-up study, mice were treated with 0 (controls), 40, 60 or 80 mg/kg/day for two weeks, and then 0, 320, 160, or 80 mg/kg/day, respectively, for 11 weeks (Cholakis et al. 1980). Definite toxic

effects were seen at the highest dose level of 320 mg/kg/day, and included hyperactivity, unscheduled deaths, and increased liver weight accompanied by hepatocellular vacuolization (males) or microgranulomas (females), and increased kidney weight with mild tubular nephrosis (males). No toxicologically significant effects were seen in mice fed the lower doses of RDX.

Levine et al. (1981) evaluated the toxicity of RDX (mixed with 3 to 10 percent HMX) administered daily in the diet of Fischer 344 rats. Groups of 10 rats per sex were given doses of 0 (control), 10, 30, 100, 300, or 600 mg/kg/day for a 13-week period. Toxicological endpoints included clinical signs, body and organ weights, food consumption, hematology, clinical chemistry, and gross and microscopic tissue morphology. Doses of 100 mg/kg/day resulted in 80 percent mortality among male rats, and 50 percent mortality in females. The higher dose levels resulted in 100 percent mortality in both sexes, with death occurring in less than three weeks. Hyperactivity, tremors and/or convulsions were observed in all animals receiving lethal doses of RDX. Some of these animals exhibited congested blood vessels and/or clotted blood in the brain at necropsy, but histopathological lesions were not found.

In the Levine et al. (1981) study, female rats appeared to be less sensitive to body weight change than male rats. The slight decrease seen in females at the 100-mg/kg/day dose was recovered by the 9th week of the study. Conversely, at test week nine, approximate weight reductions for males administered 10, 30, or 100 mg/kg/day were 6, 13, and 35 percent, respectively, and these remained as such through the end of the experiment. Males also exhibited a continual decrease in food intake at the 100-mg/kg/day dose, whereas females at this dose had reduced food intake only during the first three weeks of the test.

Levine and coworkers (1981) reported that RDX failed to alter serum cholesterol levels. Dose-related decreases in serum triglyceride levels were observed, and although slight hepatomegaly was apparent at the 100-mg/kg/day dose, microscopic lesions of the liver were not apparent. Statistically insignificant reductions in serum triglycerides were apparent at the 10-mg/kg/day dose for both males and females, although the authors felt that the 10 and 14 percent reductions, respectively, suggested biological significance. Females receiving 10 mg/kg/day dose exhibited slight but statistically significant ($P < 0.05$) leukocytosis.

4.3.2.2 Chronic Studies

Based on the results of earlier experiments (Levine et al. 1981), four dose levels were selected for use in chronic toxicity studies in an attempt to establish a no effect, marginal effect, and frank effect dose level (Levine et al. 1983). Table 15 summarizes the results of this study. Groups of 75 Fischer 344 rats per sex received RDX (mixed with 3 to 10 percent HMX) in doses of 0 (controls), 0.3, 1.5, 8.0, or 40 mg/kg/day in test diets for a period of 24 months. Rats were necropsied after 6, 12, and 24 months of treatment. Toxicological endpoints studied were the same as those used in their subchronic study described in the previous section (Levine et al. 1981). RDX at 40 mg/kg/day was

lethal to 68 percent of the males, and 36 percent of the females during the 2-yr treatment period (23 percent and 16 percent mortality, respectively, for control groups). Statistically significant ($P < 0.05$) reductions in mean survival time were seen for both sexes (35 percent and 6 percent for males and females, respectively) at the high dose level. A significant ($P < 0.05$) decrease in mean survival time (6 percent) in males at the 1.5-mg/kg/day dose level was also seen. Percent mortality for each of the doses is given in Table 16. It appears that female rats are more tolerant than males to RDX toxicity at all levels tested. The animals that died in the above study often exhibited convulsions prior to death, and those that survived treatment at the highest dose level were hyperactive to approach, and antagonistic to cage mates (Levine et al. 1983). Histological examination failed to demonstrate treatment-related lesions of the CNS.

TABLE 16. PERCENT MORTALITY IN RDX-TREATED RATS[a]

Dose Level (mg/kg/day)	Males	Females
0.0	23	16
0.3	25	13
1.5	40	17
8.0	35	19
40.0	68	36

a. Adapted from Levine et al. (1983).

In the same study, Levine et al. reported that a 20 to 30 percent reduction in body weight gain was seen in males receiving 40 mg/kg/day, and a 5 percent reduction in weight gain was seen for both sexes at the 8-mg/kg/day dose level. Females treated at the highest level often showed a 10-15 percent reduction in weight gain, but at times the weights were higher than controls.

At the 40-mg/kg/day dose, various toxic effects were observed: a slight, but significant (level of significance not reported), reduction in food intake was seen in male rats; anemia consisting of reduced hematocrit, hemoglobin, and red blood cells was seen in both sexes; thrombocytosis, hypoglycemia, hypocholesterolemia, and reductions in serum triglyceride were apparent; statistically significant ($P < 0.05$) increases in the number of cataracts in females were found; increased liver, kidney, and adrenal weights were reported for both sexes; and significant reductions (level of significance not reported) in testes weights were observed. Pathological lesions of the urinary bladder, kidneys, testes,

and spleen were considered to be treatment related for 40 mg/kg/day male rats; only splenic lesions were seen in females at this dose level (Levine et al. 1983).

As well as the decrease in mean survival time seen in male rats treated at the 1.5-mg/kg/day dose, enlarged prostate accompanied by spermatic granuloma and suppurative inflammation, and increased levels of a hemosiderin-like pigment deposited in the spleen (a secondary response to a hemolytic-type anemia) occurred.

In summary, Levine et al. (1983) report the major toxic effects of RDX to include increased mortality, anemia with secondary splenic lesions, hepatotoxicity, possible CNS involvement, cataracts, and urogenital lesions. Based on observations of adverse effects in rats administered 1.5 mg/kg/day or greater, the authors report a NOEL under the conditions reported in their study of 0.3 mg/kg/day (Levine et al. 1983).

In contrast to the results of Levine et al. (1983), Hart (1976) in an earlier study with Charles River Sprague-Dawley rats found that incorporation of RDX in the diet at a level of 0.0, 1.0, 3.1, or 10 mg/kg/day over a 2-yr period resulted in no evidence of toxicity. One hundred male and female rats per dose group were treated and examined for various clinical endpoints. Hematology, blood chemistry, urinalysis, interval gross necropsies, and organ weights and histopathological examination resulted in no important evidence of RDX toxicity.

In another study, Levine et al. (1984) evaluated the chronic toxicity and carcinogenicity of RDX (mixed with 3 to 10 percent HMX) in Charles River B6C3F1 mice when administered in the diet for 24 months. Groups of 85 rats per sex received RDX doses of 0 (controls), 1.5, 7.0, 35.0, or 175/100 mg/kg/day. The 175 mg/kg/day dose level resulted in high mortality during the first ten weeks, so the dose was lowered to 100 mg/kg/day for the remainder of the study. At this level, survival curves were similar to those for control animals. Clinical endpoints studied were the same as in the earlier studies by Levine et al. (1981, 1983). Results of the study are summarized in Table 15. The major toxic effects observed included hepatotoxicity, possible CNS involvement, and testicular degeneration. Female mice at the 175/100 mg/kg/day dose level experienced reductions in body weight gains throughout the entire study period. Male mice showed reduced weight gains during the 175 mg/kg/day study period (ten weeks), average weight gains until week 93, and then only slight reductions in weight gain through the termination of the study. The incidence of fighting wounds was greater for males at the high dose level than for other treatment levels and controls during the first year of the study, but after the first year all treatment and control groups showed high incidences of these effects. A possible treatment-related elevation in serum triglyceride levels was seen in female mice at 35 mg/kg/day, and female mice given 35 mg/kg/day, and possibly 7 mg/kg/day, exhibited elevated serum cholesterol levels. Based on these observations, an NOEL of 1.5 mg/kg/day was suggested.

4.4 GENOTOXICITY

Simmon et al. (1977) tested a number of compounds present in wastewater from munitions plants to determine the effects of water treatment on genotoxic activity. The compounds were tested using the Ames/Salmonella assay with strains of histidine-requiring Salmonella typhimurium (strains TA1535, TA1537, TA1538, TA98, and TA100), with or without activation, before and after ozonation and chlorination. RDX was not found to be genotoxic in any of the tests before or after treatment, although the authors note that the concentrations tested were very low (maximum of 100 µg/plate), and suggested the possibility of false negatives.

Stilwell et al. (1977) tested the genotoxicity of five wastewaters associated with a pilot biological treatment plant at the Holston AAP. No evidence of genotoxicity was found using the Ames/Salmonella assay with or without activation. However, even lower concentrations of RDX in the wastewater were used for the tests (0.005 to 0.52 µg/plate) than in the previous experiment (Simmon et al. 1977).

Cholakis et al. (1980) tested the genotoxicity of RDX using concentrations ranging from 0.001 to 1 mg/plate, and found no evidence of genotoxicity in the same five strains of histidine-requiring S. typhimurium listed above, with or without metabolic activation. RDX was also not genotoxic in the rat dominant lethal mutation test at doses up to 50 mg/kg/day.

Using the five previously listed strains of S. typhimurium and concentrations of 1.25 and 0.625 mg RDX/spot, Whong et al.(1980) also report that no reversions were formed. In the plate assay with induced rat liver S-9 activation and at doses up to 2.5 mg/plate, RDX gave negative results with all five strains of S. typhimurium. Similar negative results were reported by Epler (1985) using 0.01 to 0.5 mg/plate, with and without metabolic activation.

Cotruvo et al. (1977) evaluated the potential biological activity of products of ozonation in aqueous systems using short-term microbiological bioassays. Test materials were subjected to extensive ozonation in water, with 50 percent degradation of starting material attempted. The ozonated mixtures were stored to allow for decomposition of metastable products, then tested for activity. RDX was not found to react with ozone, and was not genotoxic pre- or postozonation in Salmonella or Saccharomyces test systems. A slight elevation in postozonation activity was found in Saccharomyces bioassays, but the authors report this was not dose related.

Isbister et al. (1984) studied the effects of composting on degradation and immobilization of high concentrations of TNT and RDX. Composts used in laboratory and greenhouse studies contained 45 percent chopped alfalfa hay, 45 percent horse feed, and 10 percent soil contaminated with the explosives. The initial (time zero) concentration of RDX was 9,000 mg/kg in the greenhouse compost, decreasing to 3,000 to 5,000 mg/kg after three weeks, and to 1,000 to 3,000 mg/kg after six weeks.

In the laboratory compost the initial concentration was reported as 10,000 mg/kg, dropping to about 7,000 mg/kg and 2,200 mg/kg after three and six weeks, respectively. Water extracts (leachates) of the greenhouse composts were taken at zero, three, and six weeks, and were used for testing in the Ames assay. Although RDX concentrations in the greenhouse leachate were not reported, the concentration in the water extract of the time zero RDX laboratory compost was reported to be 7.4 percent of the RDX added to the compost. In the laboratory compost, only 3.2 percent of the RDX was leachable after three weeks, and only 0.8 percent after six weeks of composting. It might be assumed that the fraction of RDX appearing in the greenhouse compost would be the same as the fraction appearing in the laboratory compost after zero, three, and six weeks. Five Salmonella test strains were used as test organisms for genotoxicity in the presence and absence of activitating enzymes (Aroclor-induced S-9 liver homogenate). Negative responses were obtained for all concentrations of RDX tested with all Salmonella strains with or without activating enzymes.

Unscheduled DNA synthesis (UDS) assays using human fibroblast (WI-38 cells) cultures were also negative (Dilley et al. 1978). At test concentrations as high as 4,000 µg/mL (the maximum feasible test concentration based on the solubility of RDX), UDS was not observed in vitro, with or without metabolic activation.

4.5 DEVELOPMENTAL/REPRODUCTIVE TOXICITY

Cholakis et al. (1980) found that RDX was not teratogenic to Charles River CD rats or New Zealand white rabbits at doses of 0, 0.2, 2.0, or 20 mg/kg/day, but at the high dose level produced maternal toxicity (primarily neurotoxicity), maternal lethality, and embryotoxicity. No adverse effects were observed at 0.2 or 2.0 mg/kg/day in rats or rabbits. The RDX used in the study was contaminated with 9 percent HMX.

A two-generation reproductive study in rats included treatment groups receiving 0 (controls), 5, 16, or 50 mg RDX/kg/day (Cholakis et al. 1980). Evidence of severe toxicity (including death, reduced body weight, and reduced feed consumption) was seen at the high dose. Although not statistically significant, reproductive effects were seen in both males and females at the high dose, including a reduced number of pregnancies and a poor survival of offspring from the pregnancies. The authors ascribed these effects to poor nutrition from the general toxicity of RDX. The lower doses produced no apparent toxicity.

4.6 ONCOGENICITY

Incorporation of RDX in the diets of rats at dose levels of 1.0, 3.1, and 10 mg/kg/day over a two-year period revealed no significant incidence of neoplasms in 16 tissues examined when compared with controls (Hart 1976). Groups of 100 rats/sex were treated at each dose level, as well as a control level, with gross necropsies and histopathologic examinations performed at 52 weeks and at termination (104 weeks).

Tissues studied included pituitary, thyroid, heart, liver, spleen, kidney, GI, and urogenital tract. Apparently no tissues from the CNS were studied for neoplasms.

A two-year feeding study by Levine et al. (1983) tested for various toxicological endpoints after 6, 12, and 24 months of treatment. Groups of 75 rats/sex received RDX doses of 0, 0.3, 1.5, 8, or 40 mg/kg/day. Ten rats/sex/dose were necropsied at the 6-, 12-, and 24-month time periods, and results of hematology, clinical chemistry, and gross and tissue morphology indicated no evidence of oncogenicity from the chronic feeding of RDX under the conditions of the study.

Levine et al. (1984) studied the carcinogenicity of RDX in Charles River B6C3F1 mice following administration of 0 (controls), 1.5, 7.0, 35.0, or 175/100 mg/kg/day for 24 months. Eighty-five mice of each sex were used in each dosage group. High mortality of mice at the 175 mg/kg/day dose caused the authors to reduce the dose to 100 mg/kg/day after the tenth week of the study. Table 17 lists the occurrence of hepatocellular carcinomas and adenomas in both sexes of mice. The frequency of hepatocellular carcinomas in female mice treated at the 35.0 and 175/100 mg/kg/day levels was found to be statistically higher ($P < 0.05$) than in controls. Although the frequency of hepatocellular adenomas in females at any dose level was not statistically different than controls, the authors found the frequency of combined hepatocellular adenomas and carcinomas to be significant ($P < 0.05$) at 7.0, 35.0, and 175/100 mg/kg/day. When historical control data were analyzed (see Table 17), combined hepatocellular adenomas and carcinomas differed significantly ($P < 0.05$) from controls only at the 35 and 175/100 mg/kg/day dose. The incidence of combined hepatocellular adenomas and carcinomas in controls was significantly lower ($P < 0.05$) than in historical controls, raising some question as to the true significance of the difference seen at the 7.0 mg/kg/day dose. The frequency of hepatocellular carcinomas and adenomas in males was not statistically different from controls at all concentrations tested. A statistically significant ($P < 0.05$) increase in malignant lymphoma of the liver was seen in only male mice (5 out of 59 mice) receiving 35 mg/kg/day, while an increased incidence of alveolar/bronchiolar carcinomas (5 out of 27 mice) was seen in the 175/100 mg/kg/day males after two years.

The data reported by Levine et al. (1984) cannot be considered conclusive evidence for the carcinogenicity of RDX for several reasons: the survival rate at 175/100 mg/kg/day was extremely low (36 percent in females and 32 percent in males compared to 70-76 percent in controls and all other dose groups for both sexes); there was no evidence of hepatocellular carcinomas in males, only females; the only indication of a dose-response is when adenomas and carcinomas are combined, with the significance attributed to the 7.0 mg/kg/day dose in question; and, the samples of RDX used in the study were contaminated with 3 to 10 percent HMX, which has not previously been tested for carcinogenicity (Ryon et al. 1984). Further, no evidence of RDX carcinogenicity was apparent in a 24-month rat study performed at the same laboratory with the same mixture of RDX and HMX (Levine et al. 1983).

TABLE 17. TWENTY-FOUR-MONTH CHRONIC ONCOGENICITY STUDY OF RDX[a] IN MICE[b,c]

Histopathologic lesion	Dose (mg/kg/day)					
	Historical control[d]	Control	1.5	7.0	35.0	175/100[e]
Males						
Hepatocellular carcinoma		13/63	20/60	16/62	18/59	6/27
Hepatocellular adenoma		8/63	6/60	1/62	7/59	7/27
Combined hepatocellular carcinoma and adenoma		21/63	26/60	17/62	25/59	13/27
Females						
Hepatocellular carcinoma	101/2469	0/65	4/62	3/64	6/64[f,g]	3/31[f]
Hepatocellular adenoma	98/2469	1/65	1/62	6/64[g]	6/64[g]	3/31
Combined hepatocellular carcinoma and adenoma	196/2469[f]	1/65[g]	5/62	9/64[f]	12/64[f,g]	6/31[f,g]

a. RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine.

b. From Levine et al. 1984.

c. All entries represent number of animals with tumors versus total number of surviving animals.

d. Historical control data only reported for females.

e. Animals were dosed with 175 mg/kg/day for 10 weeks, then dosage reduced to 100 mg/kg/day until termination of the study.

f. Statistically different from controls ($P < 0.05$).

g. Statistically different from historical controls ($P < 0.05$).

Although the results of the study by Levine et al. (1984) may be suggestive of the carcinogenicity of RDX, the absence of any other supporting data, and the limitations mentioned above, preclude the development of a carcinogenic-based risk assessment for humans.

4.7 SUMMARY

In humans and laboratory animals RDX is slowly absorbed from the stomach after ingestion and also apparently from the lungs after inhalation; there is no clinical or experimental evidence of skin absorption. In laboratory animals it appears to be extensively metabolized in the liver, does not accumulate appreciably in any tissue, and is excreted primarily in the urine or exhaled as carbon dioxide.

RDX exerts its primary toxic effect on the central nervous system, but also involves gastrointestinal and renal effects. Chronic RDX intoxication among workers in the munitions industry has been documented in several studies, exposure occurring mainly from inhalation of fine particles; because RDX is not very lipid soluble, skin absorption is very unlikely. Chronic intoxication in workers is characterized by epileptiform seizures (generalized convulsions) and unconsciousness. Convulsions may appear without warning or be preceded by one or two days of insomnia, restlessness, and irritability. Seizures are followed by temporary amnesia, disorientation, and asthenia. Acute exposures to RDX in the form of Composition C-4 have occurred following accidental ingestion or inhalation, and result in convulsions and other symptoms as described above.

Acute and chronic exposures produce a similar toxicity in animals. Pathological changes are generally nonspecific and consist of congestion in various organs, swelling and degeneration of renal tubular epithelium, fatty degeneration of the liver, and areas of hyaline degeneration of heart muscle. No pathological changes have been noted in the brain (von Oettingen et al. 1949; Sunderman 1944).

The oral LD_{50} values reported in the literature for RDX range from 44 to 300 mg/kg in the rat, indicating that RDX is moderately to highly toxic when administered to laboratory animals. There may be sex differences in response to RDX treatment. Studies evaluating the neurobehavioral toxicity of acute RDX exposure in rats found a dose-related response in schedule-controlled behavior, flavor-aversion conditioning, motor activity, and landing footspread at 12.5 mg/kg.

It has not been established whether RDX alone, or a metabolite of RDX is the effective toxic agent causing convulsions and death in mammals. However, the short time period between intravenous injection of RDX to rats and dogs, and the onset of convulsions suggests that the parent compound is responsible for the central nervous system effects.

Subchronic and chronic RDX toxicity tests have been performed in an effort to determine the mechanism of RDX toxicity, and to establish no-effect dose levels. In an attempt to ascertain whether RDX was

affecting the CNS, studies in the 1940s found that administration of nembutal, an antispasmodic drug, prevented convulsions and death in rats, and that decerebrated rats exhibited no convulsive symptoms after intraperitoneal injection of a dose of 100 mg/kg RDX (lethal in untreated rats).

Subchronic exposure of rats to RDX was found to induce time- and dose-related, biphasic, changes in brain monoamine oxidase, cholinesterase, and oxygen uptake. None of these effects were found in rats chronically dosed with 0.30 mg/kg/day for 12 weeks, thus this level is reported as an NOEL.

Chronic studies evaluated the toxicity of RDX (contaminated with 3 to 10 percent HMX) administered daily in the diet of Fischer 344 rats. During a two-year study, the major toxic effects of RDX were reported to include anemia with secondary splenic lesions, hepatotoxicity, possible CNS involvement, cataracts, and urogenital lesions. Based on observations of adverse effects in rats administered 1.5 mg/kg/day or greater, the authors report an NOEL under the conditions reported in their study of 0.3 mg/kg/day (Levine et al. 1983).

In another study evaluating the chronic toxicity and carcinogenicity of RDX in B6C3F1 hybrid mice, the major toxic effects observed after two years included hepatotoxicity, possible CNS involvement, and testicular degeneration seen at 175/100 and 35 mg/kg/day doses. A possible treatment-related elevation in serum triglyceride levels was seen in female mice at 35 mg/kg/day, and elevated serum cholesterol levels were seen in female mice at 35 mg/kg/day, and possibly 7 mg/kg/day. Based on these observations, an NOEL of 1.5 mg/kg/day was reported. This study suggests the possibility of carcinogenicity of RDX. However, the absence of an adequate dose-response curve, the high mortality rate recorded at the highest concentration tested, contamination with HMX, as well as the absence of any other supporting data, preclude the development of a carcinogenic-based risk assessment for humans based on this data. No evidence of genotoxicity or developmental/reproductive toxicity was found in various short- and long-term studies.

5. CRITERION FORMULATION

5.1 EXISTING GUIDELINES AND STANDARDS

The TLV for RDX recommended by the ACGIH (1980) is 1.5 mg/m^3, followed by the notation "skin" indicating the possibility of cutaneous absorption. However, a review of the literature indicates no evidence of skin absorption and this is supported by its low lipid solubility. The TLV value was based on the analogy of RDX to TNT and the fact that when RDX was maintained below 1.5 mg/m^3 it was effective in the prevention of injury at an Atomic Energy Commission establishment (Hyatt and Milligan 1953, as reported in Stokinger 1982). The short-term exposure limit (STEL) is 3 mg/m^3 (ACGIH 1980).

OSHA has adopted the ACGIH limit of 1.5 mg/m^3 (Stokinger 1982); the USSR has a maximum acceptable concentration of 1 mg/m^3 (ILO 1983). Based on their subchronic (drinking water) studies, Schneider et al. (1978) suggested an ingestion limit of 0.1 mg/kg/day or 2 to 3 ppm in potable water.

5.2 OCCUPATIONAL EXPOSURE

Kaplan et al. (1965) described five cases of convulsions and/or unconsciousness occurring among 26 workers employed during a four-month period in a U.S. explosives plant that processed RDX. The workers were exposed to RDX by the release of dust into the workroom air during dumping, screening, and blending of dried RDX powder and during cleanup of any spilled material. Inhalation or ingestion of the finely powdered dust was considered responsible for the toxic effects observed. All the men who became ill stated that they had failed to wear their respirators continuously and had ignored other personal hygiene directives (such as regular showers and washing of hands). Improved ventilation systems in the work areas and more strictly enforced use of personal protective measures resulted in the absence of any further cases of RDX intoxication (Kaplan et al. 1965). Several cases of RDX toxicity have been reported in the European literature, including convulsive fits, loss of consciousness, and disorientaion among workers exposed to handling or inhalation of powdered RDX during its manufacture (Barsotti and Crotti 1949, Vogel 1952). Severe irritation of the skin, oral mucous membranes, and conjuctiva was observed in a few susceptible persons (Vogel 1952).

Similar evidence of systemic intoxication was not observed at the Holston Ordnance Works during World War II (Sunderman 1944). In this plant, the Bachmann process, which requires an essentially closed system for the reaction mixture, was used to manufacture RDX. Standard procedures did not involve the handling of dry RDX. However, primary irritation and sensitization dermatitis, particularly of the face and eyelids, occurred in workers exposed to fumes during the nitration process. Studies (patch test) with 95 volunteers indicated that components of the RDX reaction mixture produced primary skin irritation after five days; however, a second application of RDX resulted in no skin reaction in any of the volunteers after two days. Patch testing with solid RDX or application of compounds present in the fumes emitted during the Bachman process failed to produce any local skin lesions similar in intensity to those observed during the manufacture of RDX. Sunderman was unable to identify the components of the fumes which were responsible for the skin lesions observed among operators at the plant.

In 1974 a cross-sectional epidemiologic study was conducted of volunteers working at five U.S. AAPs to investigate a possible association between RDX exposure and the relatively rare disease, systemic lupus erythematosis (SLE) (Hathaway and Buck 1977). The investigators attempted to identify abnormalities of the hematologic, hepatic, and renal systems, and the presence of autoimmune disease. Results of the study showed no additional cases of SLE or excess of autoimmune disease,

and no statistically significant differences in abnormalities of the hematologic, hepatic, or renal systems in employees with 8-hr time weighted exposures to RDX of up to 1.57 mg/m^3 (0.28 mg/m^3 average) compared with unexposed controls. The authors concluded that the TLV for RDX of 1.5 mg/m^3 was a safe exposure level (Hathaway and Buck 1977).

5.3 PREVIOUSLY CALCULATED CRITERIA

Sullivan et al. (1979) calculated a water criterion for the protection of aquatic organisms for RDX based on the then current USEPA proposed guidelines. The procedures in these guidelines involved methods for converting data to a common data base using various adjustment factors. Using the data of Bentley et al. (1977) exclusively, Sullivan et al. (1979) estimated a maximum freshwater concentration of 1.0 mg/L, and a 24-hr average concentration of 0.30 mg/L. This value was accepted by the USAMBRDL in an interim report to the Surgeon General's Office (U.S. Army 1982, 1983). However, it is based on a methodology which gives undue weight to very low values, and is subject to bias and anomalous behavior (Stephan et al. 1985). In addition, the current proposed USEPA guidelines (Stephan et al. 1985) are very strict in the quality control which is required in experiments used as a data base, and some of the values used by Sullivan et al. (1979) for their assessment are no longer deemed appropriate.

The USAMBRDL also calculated an interim standard for protection of human health (U.S. Army 1982, 1983). In this report, a value of 0.03 mg/L is recommended as an interim human health criterion. This value is based on a no-observed-adverse-effect-level of 1.0 mg/kg/day from a 90-day monkey-feeding study (Litton Bionetics 1974), a bioconcentration factor of 4.2 (Sullivan et al. 1979), a fish ingestion rate of 0.0187 kg/day, and an uncertainty factor of 1000. This value of 1000 was selected in the absence of valid long-term human or animal data as recommended by USEPA guidelines (USEPA 1980).

5.4 AQUATIC CRITERIA

A brief description of the methodology proposed by the U.S. Environmental Protection Agency (USEPA) for the estimation of water quality criteria suitable for the protection of aquatic life and its uses is presented in Appendix A. The aquatic criteria, as proposed by USEPA, consists of two values, a criterion maximum concentration (CMC) and a criterion continuous concentration (CCC) (Stephan et al. 1985). The CMC is equal to one-half the Final Acute Value, while the CCC is equal to the lowest of the Final Chronic Value, the Final Plant Value, or the Final Residue Value.

Data available for calculating a Final Acute Value for RDX do not meet all the requirements specified by the USEPA guidelines (Stephan et al. 1985); i.e., only six of the appropriate families of aquatic test animals have been used in acute LC_{50} tests rather than the eight families required by the guidelines (see Appendix A). However, since the

data generated by these toxicity tests are uniform in their assessment of the degree of toxicity of RDX, a freshwater Final Acute Value was estimated using the formulae provided in the USEPA guidelines (Stephan et al. 1985).

The steps used for calculating the Final Acute Value are shown in Table 18. Since eight families of aquatic test animals were used in the acute toxicity testing, even though they do not represent the necessary eight families required by the Guidelines, the value of N is taken as equal to eight. A Final Acute Value of 5.1821 mg/L is estimated. It should be emphasized that this value represents an interim value because, as noted above, in accordance with the USEPA Guidelines (Stephan et al. 1985), a final freshwater animal acute value cannot be calculated until two additional families are tested for RDX toxicity.

According to USEPA Guidelines for water quality criteria, acute and chronic flow-through tests using measured concentrations for three species of organisms are required to estimate an acute/chronic ratio. Results from such tests are not available and an acute/chronic ratio for RDX cannot be determined.

Bentley et al. (1977) studied the aquatic toxicity of RDX to Microcystis aeruginosa, Anabeana flos-aquae, Selenastrum capricornutum, and Navicula pelliculosa. In general, the species M. aeruginosa, A. flos-aquae, and N. pelliculosa showed minimal (1 to 3 percent) decreases in cell growth and chlorophyll a content at all concentrations below 10 mg/L, and only 4 to 11 percent decreases at the 10 mg/L concentration. RDX had a more pronounced effect on S. capricornutum, with decreases of 17 percent in cell density and chlorophyl a content at the 3.2 mg/L concentration, and 2 to 16 percent decreases at the lower concentrations tested. Based on their analysis, Bentley et al. (1977) found the changes in growth or chlorophyll a content to be insignificant, and reported 96-hr EC_{50} values greater than 32 mg/L for all the algal species studied. Due to the questions that Sullivan et al. (1979) have raised regarding the Bentley et al. statistical analysis of the algal toxicity data, and the absence of a definitive EC_{50} for growth or chlorophyll a content, the data does not seem appropriate for use in selecting a Final Plant Value.

Bioconcentration studies for RDX indicate that it is only slightly accumulated in tissues of aquatic organisms. Bioaccumulation factors range from 1.4 to 4.7 in the edible tissue of freshwater fish (see Table 11). Elimination of all RDX from the tissues at low dose exposure occurred after 14 days in bluegill sunfish and channel catfish but did not occur totally in fathead minnows. At a higher dose, fathead minnows and channel catfish eliminated 70 to 87 percent of the accumulated RDX, but virtually no elimination occurred from either muscle or viscera in bluegill sunfish (Bentley et al. 1977). No maximum permissible tissue concentration is available for RDX, nor are there any chronic wildlife feeding or field studies to estimate acceptable dietary intake. Therefore, no Final Residue Value can be calculated.

TABLE 18. CALCULATIONS FOR FINAL ACUTE VALUE (FAV) OF RDX[a,b]

Rank (R)	GMAV[c]	ln GMAV[d]	(ln GMAV)^2	P = R/(N+1)[e,f]	$\sqrt{P}$
4	7.3	1.9879	3.9517	0.4444	0.6667
3	6.75	1.9095	3.6462	0.3333	0.5774
2	6.4	1.8563	3.4458	0.2222	0.4714
1	5.6	1.7228	2.9680	0.1111	0.3333
Sum:		7.4764	14.0116	1.1111	2.04875

a. RDX = hexahydro-1,3,5-trinitro-1,3,5-triazine.
b. Based on calculation methods discussed in Stephan et al. (1985).
c. GMAV = genus mean acute value in mg/L.
d. ln GMAV = natural log of GMAV.
e. N = 8.
f. P = probability for each GMAV; R = rank of four lowest GMAVs.

$$S^2 = \frac{\Sigma((\ln GMAV)^2) - ((\Sigma(\ln GMAV))^2/4)}{\Sigma(P) - ((\Sigma(\sqrt{P}))^2/4},$$

$$L = (\Sigma(\ln GMAV) - S(\Sigma(\sqrt{P}))/4,$$

$$A = S(\sqrt{0.05}) + L,$$

$$FAV = e^A,$$

$$S^2 = \frac{14.0117 - (7.4765)^2/4}{1.11110 - (2.04875)^2/4} = 0.60212;\ S = 0.7760,$$

$$L = (7.4765 - (0.7760)(2.04875))/4 = 1.4717,$$

$$A = (0.7760)(\sqrt{0.05}) + (1.4717) = 1.6452, \text{ and}$$

$$FAV = e^{1.6452} = 5.1821.$$

In summary, CMC is equal to one-half the Final Acute Value while the CCC is equal to the Chronic Value, the Final Plant Value, or the Final Residue Value, whichever is lowest. According to USEPA Guidelines (Stephan et al. 1985), the Final Chronic Value is equal to the Final Acute Value divided by the Final Acute/Chronic Ratio. The minimum data base specified by the USEPA, in their guidelines for estimating water quality criteria, is not available for RDX, and thus no CMC or CCC can be derived.

5.5 HUMAN HEALTH CRITERIA

There is not sufficient data from human or animal tests to quantitatively prove that RDX is carcinogenic in mammals or to derive a water quality criterion based on a nonthreshold low-dose extrapolation procedure. There are also no available human studies suitable for estimating a maximum daily oral intake producing no detectable adverse effects. Therefore, data for the calculation of a human health criterion comes from the long-term chronic study of Levine et al. (1983), who evaluated the toxicity of RDX administered daily in the diet of rats for a period of 24 months. Four dose levels were selected for use in chronic toxicity studies in an attempt to establish a no effect, marginal effect, and frank effect dose level. Toxicological endpoints included clinical signs, body and organ weights, food consumption, hematology, clinical chemistry, and gross and microscopic tissue morphology. No evidence of carcinogenicity was reported in this study.

RDX at 40 mg/kg/day was lethal to 68 percent of the males and 36 percent of the females during the 2-yr treatment period, and statistically significant reductions ($P < 0.05$) in mean survival time were seen for both sexes. At this level, anemia consisting of reduced hematocrit, hemoglobin, and red blood cells was seen in both sexes; thrombocytosis, hypoglycemia, hypocholesterolemia, and reductions in serum triglyceride were apparent; statistically significant increases in the number of cataracts in females were found; increased liver, kidney, and adrenal weights were reported for both sexes; and significant reductions in testes weights were observed. The dose of 40 mg/kg/day appears to be a well-defined "frank effect level." A significant decrease in mean survival time (6 percent) in males at the 1.5-mg/kg/day dose level was also seen, as well as enlarged prostate accompanied by spermatic granuloma and suppurative inflammation, and increased levels of a hemosiderin-like pigment deposited in the spleen (a secondary response to a hemolytic-type anemia). Based on the observation of adverse effects seen at 1.5 mg/kg/day, the authors report a no observed effects level (NOEL) under the conditions reported in their study of 0.3 mg/kg/day. It should be pointed out that the RDX administered in the study was contaminated with 3 to 10 percent HMX, and it is not known to what extent this may have affected the results of the experiment.

Brown (1975) found RDX to induce time- and dose-related, biphasic, changes in brain monoamine oxidase, cholinesterase, and oxygen uptake in rats during subchronic (12 week) oral feeding studies. Based on his results, Brown also reported 0.3 mg/kg/day as an NOEL.

The methodology outlined by the USEPA for estimation of a water quality criterion for protection of human health (USEPA 1980) is summarized in Appendix B. Using the NOEL of 0.3 mg/kg/day reported by Levine et al. (1983), and an uncertainty factor of 100, an acceptable daily intake for a 70-kg human is calculated to be 0.21 mg/day:

$$\text{ADI (mg/day)} = \frac{70 \text{ kg} \times \text{NOEL (mg/kg/day)}}{\text{uncertainty factor}}$$

The uncertainty factor of 100 was selected because the results are from valid long-term feeding studies on experimental animals in which a well-defined LOAEL and NOEL exist.

The equation for calculating the human health criterion for RDX given an ADI is

$$C = \frac{\text{ADI} - (\text{DT} + \text{IN})}{2 \text{ L/day} + (0.0065 \text{ kg/day} \times \text{BCF} \times 1 \text{ L/kg})} ,$$

where

C = water quality criterion;

ADI = acceptable daily intake, 0.21 mg/day;

DT = dietary non-fish intake, assumed to be 0;

IN = inhalation intake, assumed to be 0;

2L = daily water intake in liters;

0.0065 = daily dietary fish intake;

BCF = bioconcentration factor, assumed to be 4.7 (see Table 11); and

1L/kg = unit conversion factor.

Using the methodology of the USEPA (1980), an ambient water quality criterion for the protection of human health and sensitive populations of 103 µg/L is proposed. Although a water quality criterion to protect aquatic organisms and their uses cannot be derived, a Final Acute Value of 5.1821 mg/L for aquatic organisms has been estimated. Based on this limited information, it appears that the concentration of 103 µg/L proposed as a human health criterion will probably be protective of most aquatic organisms and their uses.

5.6 RESEARCH RECOMMENDATIONS

The following research recommendations are intended to fill the data gaps necessary to meet the USEPA requirements for generating human and aquatic water quality criteria.

1. A definitive 2-yr feeding study to adequately determine whether RDX is carcinogenic. In light of the problems associated with the

study of Levine et al. (1984) it is recommended that the 2-yr mouse study be repeated. Dose levels should be well below lethal levels to ensure that mortality rates are similar between controls and test animals, and uncontaminated RDX should be used, if available. If a second species is tested, it is suggested that a rat strain other than that previously tested by Levine et al. (1983), or the hamster, be utilized.

2. Acute toxicity tests for a family in any order of insect or any phylum not represented, e.g., the mayfly nymph Hexagenia sp.; and a family in a phylum other than Arthropoda or Chordata, e.g., the aquatic oligochaete, Lumbriculus variegatus.

3. Chronic flow-through tests using measured concentrations for at least three species of aquatic animals, provided that one is an invertebrate species and one a sensitive freshwater species. These tests could include life-stage tests with Pimephales promelas and Salmo gairdneri, and a 21-day life-cycle test with Daphnia magna.

4. Acute flow-through tests using measured concentrations for the three species of organisms for which chronic toxicity tests have been performed in order to fulfill the requirements for calculating the three acute-chronic ratios.

5. Data on the significance of residues in aquatic species, in particular derivation of a maximum permissible tissue concentration for RDX.

6. Definitive steady-state or 28-day bioaccumulation studies for RDX in aquatic tissues.

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7. GLOSSARY

AAP	Army Ammunition Plant
ACGIH	American Conference of Governmental Industrial Hygienists
ADI	Acceptable daily intake
BCF	Bioconcentration factor
BOD	Biological oxygen demand
C-4	A plastic explosive (91 percent RDX)
CCC	Criterion continuous concentration
CMC	Criterion maximum concentration
CNS	Central Nervous System
COD	Chemical oxygen demand
CSF	Cerebrospinal fluid
DMSO	Dimethylsulfoxide
EC_{50}	Effective concentration causing 50 percent death (based on immobilization)
ED_{50}	Dose causing a response that is 50 percent of the control value
FAV	Final acute value
FCV	Final chronic value
FPV	Final plant value
FRV	Final residue value
GI	Gastrointestinal
GLC	Gas-liquid chromatography
HMX	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
HPLC	High pressure liquid chromatography
LC_{50}	Lethal concentration causing 50 percent death
LD_{50}	Lethal dose causing 50 percent death

LOAEL	Lowest observed adverse effect level
LOEL	Lowest observed effect level
MAO	Monoamine oxidase
MFO	Mixed function oxidase
NOAEL	No observed adverse effect level
NOEL	No observed effect level
ppm	Parts per million
RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine
SGOT	Serum glutamic oxalacetic transaminase
SLE	Systemic lupus erythematosis
STEL	Short-term exposure limit
TLC	Thin layer chromatography
TLV	Threshold limit value
TNT	Trinitrotoluene
USAMBRDL	U.S. Army Medical Bioengineering Research and Development Laboratory
USEPA	U.S. Environmental Protection Agency
UV	Ultraviolet
VI	Variable interval
VR	Variable ratio

APPENDIX A:

SUMMARY OF USEPA METHODOLOGY FOR DERIVING NUMERICAL WATER QUALITY CRITERIA FOR THE PROTECTION OF AQUATIC ORGANISMS AND THEIR USES

The following summary is a condensed version of the 1985 final US Environmental Protection Agency (USEPA) guidelines for calculating a water quality criteria to protect aquatic life and is slanted towards the specific regulatory needs of the US Army (e.g., discussion of saltwater aspects of the criteria calculation are not included). The guidelines are the most recent document outlining the required procedures and were written by the following researchers from the USEPA's regional research laboratories: C.E. Stephan, D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman, and W.A. Brungs. For greater detail on individual points consult Stephan et al. (1985).

1. INTRODUCTION

The Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses describe an objective, internally consistent, and appropriate way of estimating national criteria. Because aquatic life can tolerate some stress and occasional adverse effects, protection of all species all of the time was not deemed necessary. If acceptable data are available for a large number of appropriate taxa from a variety of taxonomic and functional groups, a reasonable level of protection should be provided if all except a small fraction are protected, unless a commercially, recreationally, or socially important species was very sensitive. The small fraction is set at 0.05 because other fractions resulted in criteria that seemed too high or too low in comparison with the sets of data from which they were calculated. Use of 0.05 to calculate a Final Acute Value does not imply that this percentage of adversely affected taxa should be used to decide in a field situation whether a criterion is appropriate.

To be acceptable to the public and useful in field situations, protection of aquatic organisms and their uses should be defined as prevention of unacceptable long-term and short-term effects on (1) commercially, recreationally, and socially important species and (2) (a) fish and benthic invertebrate assemblages in rivers and streams and (b) fish, benthic invertebrate, and zooplankton assemblages in lakes, reservoirs, estuaries, and oceans. These national guidelines have been developed on the theory that effects which occur on a species in appropriate laboratory tests will generally occur on the same species in comparable field situations.

Numerical aquatic life criteria derived using these national guidelines are expressed as two numbers, so that the criteria can more accurately reflect toxicological and practical realities. The combination of a maximum concentration and a continuous concentration is designed to provide adequate protection of aquatic life and its uses from acute and chronic toxicity to animals, toxicity to plants, and

bioaccumulation by aquatic organisms without being as restrictive as a one-number criterion would have to be in order to provide the same degree of protection.

Criteria produced by these guidelines should be useful for developing water quality standards, mixing zone standards, and effluent standards. Development of such standards may have to consider additional factors such as social, legal, economic, and additional biological data. It may be desirable to derive site-specific criteria from these national criteria to reflect local conditions (USEPA 1982). The two factors that may cause the most difference between the national and site-specific criteria are the species that will be exposed and the characteristics of the water.

Criteria should provide reasonable and adequate protection with only a small possibility of considerable overprotection or underprotection. It is not enough that a criterion be the best estimate obtainable using available data; it is equally important that a criterion be derived only if adequate appropriate data are available to provide reasonable confidence that it is a good estimate. Thus, these guidelines require that certain data be available if a criterion is to be derived. If all the required data are not available, usually a criterion should not be derived; however, availability of all required data does not ensure that a criterion can be derived. The amount of guidance in these national guidelines is significant, but much of it is necessarily qualitative rather than quantitative; much judgement will be required to derive a water quality criterion for aquatic life. All necessary decisions should be based on a thorough knowledge of aquatic toxicology and an understanding of these guidelines and should be consistent with the spirit of these guidelines - which is to make best use of all available data to derive the most appropriate criterion.

2. DEFINITION OF MATERIAL OF CONCERN

1. Each separate chemical that does not ionize significantly in most natural bodies of water should be considered a separate material, except possibly for structurally similar organic compounds that only exist in large quantities as commercial mixtures of the various compounds and apparently have similar biological, chemical, physical, and toxicological properties.

2. For chemicals that do ionize significantly, all forms that would be in chemical equilibrium should usually be considered one material. Each different oxidation state of a metal and each different nonionizable covalently bonded organometallic compound should usually be considered a separate material.

3. Definition of the material should include an operational analytical component. It is also necessary to reference or

describe analytical methods that the term is intended to denote. Primary requirements of the operational analytical component is that it be appropriate for use on samples of receiving water, that it be compatible with toxicity and bioaccumulation data without making extrapolations that are too hypothetical, and that it rarely result in underprotection of aquatic life and its uses.

NOTE: Analytical chemistry of the material may have to be considered when defining the material or when judging acceptability of some toxicity tests, but a criterion should not be based on sensitivity of an analytical method. When aquatic organisms are more sensitive than analytical techniques, the proper solution is to develop better analytical methods, not to underprotect aquatic life.

3. COLLECTION OF DATA

1. Collect all available data on the material concerning (a) toxicity to, and bioaccumulation by, aquatic animals and plants; (b) FDA action levels (FDA Guidelines Manual); and (c) chronic feeding studies and long-term field studies with wildlife that regularly consume aquatic organisms.

2. All data used should be available in typed, dated and signed hardcopy with enough supporting information to indicate that acceptable test procedures were used and the results should be reliable.

3. Questionable data, whether published or not, should not be used.

4. Data on technical grade materials may be used if appropriate, but data on formulated mixtures and emulsifiable concentrates of the test material should not be used.

5. For some highly volatile, hydrolyzable, or degradable materials it may be appropriate to only use results of flow-through tests in which concentration of test material in test solutions were measured using acceptable analytical methods.

6. Do not use data obtained using brine shrimp, species that do not have reproducing wild populations in North America, or organisms that were previously exposed to significant concentrations of the test material or other contaminants.

4. REQUIRED DATA

1. Results of acceptable acute tests (see Section 5) with freshwater animals in at least eight different families such that all of the following are included:

   a. the family Salmonidae in the class Osteichthyes;

   b. a second family (preferably an important warmwater species) in the class Osteichthyes (e.g., bluegill, fathead minnow, or channel catfish);

   c. a third family in the phylum Chordata (e.g, fish or amphibian);

   d. a planktonic crustacean (e.g, cladoceran or copepod);

   e. a benthic crustacean (e.g, ostracod, isopod, or amphipod);

   f. an insect (e.g., mayfly, midge, stonefly);

   g. a family in a phylum other than Arthropoda or Chordata (e.g, Annelida or Mollusca); and

   h. a family in any order of insect or any phylum not represented.

2. Acute-chronic ratios (see Section 7) for species of aquatic animals in at least three different families provided that of the three species at least (a) one is a fish, (b) one is an invertebrate, and (c) one is a sensitive freshwater species.

3. Results of at least one acceptable test with a freshwater alga or a chronic test with a freshwater vascular plant (see Section 9). If plants are among the aquatic organisms that are most sensitive to the material, results of a test with a plant in another phylum (division) should be available.

4. At least one acceptable bioconcentration factor determined with an appropriate aquatic species, if a maximum permissible tissue concentration is available (see Section 10).

If all required data are available, a numerical criterion can usually be derived, except in special cases. For example, if a criterion is to be related to a water quality characteristic (see Sections 6 and 8), more data will be necessary. Similarly if all required data are not available a numerical criterion should not be derived except in special cases. For example, even if not enough acute and chronic data are available, it

may be possible to derive a criterion if the data clearly indicate that the Final Residue Value would be much lower than either the Final Chronic Value or the Final Plant Value. Confidence in a criterion usually increases as the amount of data increases. Thus, additional data are usually desirable.

5. FINAL ACUTE VALUE

1. The Final Acute Value (FAV) is an estimate of the concentration of material corresponding to a cumulative probability of 0.05 in the acute toxicity values for the genera with which acute tests have been conducted on the material. However, in some cases, if the Species Mean Acute Value (SMAV) of an important species is lower than the calculated FAV, then that SMAV replaces the FAV to protect that important species.

2. Acute toxicity tests should have been conducted using acceptable procedures (e.g., ASTM Standard E 724 or 729).

3. Generally, results of acute tests in which food was added to the test solution should not be used, unless data indicate that food did not affect test results.

4. Results of acute tests conducted in unusual dilution water, e.g., dilution water containing high levels of total organic carbon or particulate matter (higher than 5 mg/L) should not be used, unless a relationship is developed between toxicity and organic carbon or unless data show that organic carbon or particulate matter, etc. do not affect toxicity.

5. Acute values should be based on endpoints which reflect the total adverse impact of the test material on the organisms used in the tests. Therefore, only the following kinds of data on acute toxicity to freshwater aquatic animals should be used:

    a. Tests with daphnids and other cladocerans should be started with organisms <24 hr old and tests with midges should be started with second- or third-instar larvae. The result should be the 48-hr EC_{50} based on percentage of organisms immobilized plus percentage of organisms killed. If such an EC_{50} is not available from a test, the 48-hr LC_{50} should be used in place of the desired 48-hr EC_{50}. An EC_{50} or LC_{50} of longer than 48 hr can be used provided animals were not fed and control animals were acceptable at the end of the test.

b. The result of tests with all other aquatic animal species should be the 96-hr EC_{50} value based on percentage of organisms exhibiting loss of equilibrium plus percentage of organisms immobilized plus percentage of organisms killed. If such an EC_{50} value is not available from a test, the 96-hr LC_{50} should be used in place of the desired EC_{50}.

c. Tests with single-cell organisms are not considered acute tests, even if the duration was $\leq$96 hr.

d. If the tests were conducted properly, acute values reported as greater than values and those acute values which are above solubility of the test material are acceptable.

6. If the acute toxicity of the material to aquatic animals has been shown to be related to a water quality characteristic (e.g., total organic carbon) for freshwater species, a Final Acute Equation should be derived based on that characteristic.

7. If the data indicate a that one or more life stages are at least a factor of 2 times more resistant than one or more other life stages of the same species, the data for the more resistant life stages should not be used in the calculation of the SMAV because a species can only be considered protected from acute toxicity if all life stages are protected.

8. Consider the agreement of the data within and between species. Questionable results in comparison to other acute and chronic data for the species and other species in the same genus probably should not be used.

9. For each species for which at least one acute value is available, the SMAV should be calculated as the geometric mean of all flow-through test results in which the concentration of test material were measured. For a species for which no such result is available, calculate the geometric mean of all available acute values, i.e., results of flow-through tests in which the concentrations were not measured and results of static and renewal tests based on initial total concentrations of test material.

   NOTE: Data reported by original investigators should not be rounded off and at least four significant digits should be retained in intermediate calculations.

10. For each genus for which one or more SMAV is available, calculate the Genus Mean Acute Value (GMAV) as the geometric mean of the SMAVs.

11. Order the GMAVs from high to low and assign ranks (R) to the GMAVs from "1" for the lowest to "N" for the highest. If two or more GMAVs are identical, arbitrarily assign them successive ranks.

12. Calculate the cumulative probability (P) for each GMAV as R/(N+1).

13. Select the four GMAVs which have cumulative probabilities closest to 0.05 (if there are <59 GMAVs, these will always be the four lowest GMAVs).

14. Using the selected GMAVs and Ps, calculate

$$S^2 = \frac{\Sigma((\ln GMAV)^2) - ((\Sigma(\ln GMAV))^2/4)}{\Sigma(P) - ((\Sigma(\sqrt{P}))^2/4}$$

$$L = (\Sigma(\ln GMAV) - S(\Sigma(\sqrt{P})))/4$$

$$A = S(\sqrt{0.05}) + L$$

$$FAV = e^A$$

15. If for an important species, such as a recreationally or commercially important species, the geometric mean of acute values from flow-through tests in which concentrations of test material were measured is lower than the FAV, then that geometric mean should be used as the FAV.

16. Go to Section 7.

6. FINAL ACUTE EQUATION

1. When enough data show that acute toxicity to two or more species is similarly related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.

2. For each species for which comparable acute toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of acute toxicity values on values of the water quality characteristic.

3. Decide whether the data for each species is useful, considering the range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used.

4. Individually for each species calculate the geometric mean of the acute values and then divide each of the acute values for a species by the mean for the species. This normalizes the acute values so that the geometric mean of the normalized values for each species individually and for any combination of species is 1.0

5. Similarly normalize the values of the water quality characteristic for each species individually.

6. Individually for each species perform a least squares regression of the normalized acute toxicity values on the corresponding normalized values of of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 2. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

7. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized acute values on the corresponding normalized values of the water quality characteristic to obtain the pooled acute slope (V) and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

8. For each species calculate the geometric mean (W) of the acute toxicity values and the geometric mean (X) of the related values of the water quality characteristic (calculated in 4. and 5. above).

9. For each species calculate the logarithmic intercept (Y) of the SMAV at a selected value (Z) of the water quality characteristic using the equation: $Y = \ln W - V(\ln X - \ln Z)$.

10. For each species calculate the SMAV using: $SMAV = e^{Y}$.

11. Obtain the FAV at Z by using the procedure described in Section 5. (No. 10-14).

12. If the SMAV for an important species is lower than the FAV at Z, then that SMAV should be used as the FAV at Z.

13. The Final Acute Equation is written as: FAV = $e^{(V[\ln(\text{water quality characteristic}) + \ln A - V[\ln Z])}$, where V = pooled acute slope and A = FAV at Z. Because V, A, and Z are known, the FAV can be calculated for any selected value of the water quality characteristic.

7. FINAL CHRONIC VALUE

1. Depending on available data, the Final Chronic Value (FCV) might be calculated in the same manner as the FAV or by dividing the FAV by the Final Acute-Chronic Ratio.

   NOTE: Acute-chronic ratios and application factors are ways of relating acute and chronic toxicities of a material to aquatic organisms. Safety factors are used to provide an extra margin of safety beyond known or estimated sensitivities of aquatic organisms. Another advantage of the acute-chronic ratio is that it should usually be greater than one; this should avoid confusion as to whether a large application factor is one that is close to unity or one that has a denominator that is much greater than the numerator.

2. Chronic values should be based on results of flow-through (except renewal is acceptable for daphnids) chronic tests in which concentrations of test material were properly measured at appropriate times during testing.

3. Results of chronic tests in which survival, growth, or reproduction in controls was unacceptably low should not be used. Limits of acceptability will depend on the species.

4. Results of chronic tests conducted in unusual dilution water should not be used, unless a relationship is developed between toxicity and the unusual characteristic or unless data show the characteristic does not affect toxicity.

5. Chronic values should be based on endpoints and exposure durations appropriate to the species. Therefore, only results of the following kinds of chronic toxicity tests should be used:

a. Life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Tests with fish should begin with embryos or newly hatched young <48 hr old, continue through maturation and reproduction, and should end not <24 days (90 days for salmonids) after the hatching of the next generation. Tests with daphnids should begin with young <24 hr old and last for not <21 days. For fish, data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability. For daphnids, data should be obtained and analyzed on survival and young per female.

b. Partial life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Partial life-cycle tests are allowed with fish species that require more than a year to reach sexual maturity, so that all major life stages can be exposed to the test material in less than 15 months. Exposure to the test material should begin with juveniles at least 2 months prior to active gonadal development, continue through maturation and reproduction, and should end not <24 days (90 days for salmonids) after the hatching of the next generation. Data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability.

c. Early life-stage toxicity tests consisting of 28- to 32-day (60 days posthatch for salmonids) exposures of early life stages of a species of fish from shortly after fertilization through embryonic, larval, and early juvenile development. Data should be obtained on growth and survival.

NOTE: Results of an early life-stage test are used as predictors of results of life-cycle and partial life-cycle tests with the same species. Therefore, when results of a life-cycle or partial life-cycle test are available, results of an early life-stage test with the same species should not be used. Also, results of early life-stage tests in which the incidence of mortalities or abnormalities increased substantially near the end of the test should not be used because results of such tests may be poor estimates of results of a comparable life-cycle or partial life-cycle test.

6. A chronic value may be obtained by calculating the geometric mean of lower and upper chronic limits from a chronic test or by analyzing chronic data using regression analysis. A lower chronic limit is the highest tested concentration (a) in an acceptable chronic test, (b) which did not cause an unacceptable amount of an adverse effect on any specified biological measurements, and (c) below which no tested concentration caused such an unacceptable effect. An upper chronic limit is the lowest tested concentration (a) in an acceptable chronic test, (b) which did cause an unacceptable amount of an adverse effect on one or more of specified biological measurements, and (c) above which all tested concentrations caused such an effect.

7. If chronic toxicity of material to aquatic animals appears to be related to a water quality characteristic, a Final Chronic Equation should be derived based on that water quality characteristic. Go to Section 8.

8. If chronic values are available for species in eight families as described in Section 4 (No. 1), a Species Mean Chronic Value (SMCV) should be calculated for each species for which at least one chronic value is available by calculating the geometric mean of all chronic values for the species and appropriate Genus Mean Chronic Values should be calculated. The FCV should then be obtained using procedures described in Section 5 (No. 10-14). Then go to Section 7 (No. 13).

9. For each chronic value for which at least one corresponding appropriate acute value is available, calculate an acute-chronic ratio, using for the numerator the geometric mean of results of all acceptable flow-through (except static is acceptable for daphnids) acute tests in the same dilution water and in which concentrations were measured. For fish, the acute test(s) should have been conducted with juveniles. Acute test(s) should have been part of the same study as the chronic test. If acute tests were not conducted as part of the same study, acute tests conducted in the same laboratory and dilution water may be used. If acute tests were not conducted as part of the same study, acute tests conducted in the same dilution water but a different laboratory may be used. If such acute tests are not available, an acute-chronic ratio should not be calculated.

10. For each species, calculate the species mean acute-chronic ratio as the geometric mean of all acute-chronic ratios for that species.

11. For some materials the acute-chronic ratio is about the same for all species, but for other materials the ratio increases or decreases as the SMAV increases. Thus, the Final Acute-Chronic Ratio can be obtained in three ways, depending on the data.

    a. If the species mean acute-chronic ratio increases or decreases as the SMAV increases, the final Acute-Chronic Ratio should be calculated as the geometric mean of all species whose SMAVs are close to the FAV.

    b. If no major trend is apparent and the acute-chronic ratios for a number of species are within a factor of ten, the Final Acute-Chronic Ratio should be calculated as the geometric mean of all species mean acute-chronic ratios for both freshwater and saltwater species.

    c. If the most appropriate species mean acute-chronic ratios are <2.0, and especially if they are <1.0, acclimation has probably occurred during the chronic test. Because continuous exposure and acclimation cannot be assured to provide adequate protection in field situations, the Final Acute-Chronic Ratio should be set at 2.0 so that the FCV is equal to the Criterion Maximum Concentration.

    If the acute-chronic ratios do not fit one of these cases, a Final Acute-Chronic Ratio probably cannot be obtained, and a FCV probably cannot be calculated.

12. Calculate the FCV by dividing the FAV by the Final Acute-Chronic Ratio.

13. If the SMAV of an important species is lower than the calculated FCV, then that SMCV should be used as the FCV.

14. Go to Section 9.

8. FINAL CHRONIC EQUATION

1. A Final Chronic Equation can be derived in two ways. The procedure described in this section will result in the chronic slope being the same as the acute slope.

    a. If acute-chronic ratios for enough species at enough values of the water quality characteristics indicate that the acute-chronic ratio is probably the same for all species and independent of the water quality

characteristic, calculate the Final Acute-Chronic Ratio as the geometric mean of the species mean acute-chronic ratios.

b. Calculate the FCV at the selected value Z of the water quality characteristic by dividing the FAV at Z by the Final Acute-Chronic Ratio.

c. Use V = pooled acute slope as L = pooled chronic slope.

d. Go to Section 8, No. 2, item m.

2. The procedure described in this section will usually result in the chronic slope being different from the acute slope.

a. When enough data are available to show that chronic toxicity to at least one species is related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.

b. For each species for which comparable chronic toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of chronic toxicity values on values of the water quality characteristic.

c. Decide whether data for each species is useful, taking into account range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used. If a useful chronic slope is not available for at least one species or if the slopes are too dissimilar or if data are inadequate to define the relationship between chronic toxicity and water quality characteristic, return to Section 7 (No. 8), using results of tests conducted under conditions and in water similar to those commonly used for toxicity tests with the species.

d. For each species calculate the geometric mean of the available chronic values and then divide each chronic value for a species by the mean for the species.

This normalizes the chronic values so that the geometric mean of the normalized values for each species and for any combination of species is 1.0.

e. Similarly normalize the values of the water quality characteristic for each species individually.

f. Individually for each species perform a least squares regression of the normalized chronic toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 1. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

g. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized chronic values on the corresponding normalized values of the water quality characteristic to obtain the pooled chronic slope (L) and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

h. For each species calculate the geometric mean (M) of toxicity values and the geometric mean (P) of related values of the water quality characteristic.

i. For each species calculate the logarithm (Q) of the SMCVs at a selected value (Z) of the water quality characteristic using the equation: $Q = \ln M - L(\ln P - \ln Z)$.

j. For each species calculate a SMCV at Z as the antilog of Q ($SMCV = e^{Q}$).

k. Obtain the FCV at Z by using the procedure described in Section 5 (No. 10-14).

l. If the SMCV at Z of an important species is lower than the calculated FCV at Z, then that SMCV should be used as the FCV at Z.

m. The Final Chronic Equation is written as: $FCV = e^{(L[\ln(\text{water quality characteristic})] + \ln S - L[\ln Z])}$, where L = mean chronic slope and S = FCV at Z.

9. FINAL PLANT VALUE

1. Appropriate measures of toxicity of the material to aquatic plants are used to compare relative sensitivities of aquatic plants and animals. Although procedures for conducting and interpreting results of toxicity tests with plants are not well developed, results of such tests usually indicate that criteria which adequately protect aquatic animals and their uses also protect aquatic plants and their uses.

2. A plant value is the result of any test conducted with an alga or an aquatic vascular plant.

3. Obtain the Final Plant Value by selecting the lowest result obtained in a test on an important aquatic plant species in which concentrations of test material were measured and the endpoint is biologically important.

10. FINAL RESIDUE VALUE

1. The Final Residue Value (FRV) is intended to (a) prevent concentrations in commercially or recreationally important aquatic species from exceeding applicable FDA action levels and (b) protect wildlife, including fish and birds, that consume aquatic organisms from demonstrated unacceptable effects. The FRV is the lowest of residue values that are obtained by dividing maximum permissible tissue concentrations by appropriate bioconcentration or bioaccumulation factors. A maximum permissible tissue concentration is either (a) a FDA action level (FDA administrative guidelines) for fish oil or for the edible portion of fish or shellfish or (b) a maximum acceptable dietary intake (ADI) based on observations on survival, growth, or reproduction in a chronic wildlife feeding study or a long-term wildlife field study. If no maximum permissible tissue concentration is available, go to Section 11., because a Final Residue Value cannot be derived.

2. Bioconcentration Factors (BCFs) and Bioaccumulation Factors (BAFs) are the quotients of the concentration of a material in one or more tissues of an aquatic organism divided by the average concentration in the solution to which the organism has been exposed. A BCF is intended to account only for net uptake directly from water, and thus almost has to be measured in a laboratory test. A BAF is intended to account for net uptake from both food and water in a real-world situation, and almost has to be measured in a field situation in which predators accumulate the material directly from

water and by consuming prey. Because so few acceptable BAFs are available, only BCFs will be discussed further, but an acceptable BAF can be used in place of a BCF.

3. If a maximum permissible tissue concentration is available for a substance (e.g, parent material or parent material plus metabolite), the tissue concentration used in BCF calculations should be for the same substance. Otherwise the tissue concentration used in the BCF calculation should be that of the material and its metabolites which are structurally similar and are not much more soluble in water than the parent material.

   a. A BCF should be used only if the test was flow-through, the BCF was calculated based on measured concentrations of test material in tissue and in the test solution, and exposure continued at least until either apparent steady-state (BCF does not change significantly over a period of time, such as two days or 16 percent of exposure duration, whichever is longer) or 28 days was reached. The BCF used from a test should be the highest of (a) the apparent steady-state BCF, if apparent steady-state was reached; (b) highest BCF obtained, if apparent steady-state was not reached; and (c) projected steady-state BCF, if calculated.

   b. Whenever a BCF is determined for a lipophilic material, percentage of lipids should also be determined in the tissue(s) for which the BCF is calculated.

   c. A BCF obtained from an exposure that adversely effected the test organisms may be used only if it is similar to that obtained with unaffected individuals at lower concentrations that did cause effects.

   d. Because maximum permissible tissue concentrations are rarely based on dry weights, a BCF calculated using dry tissue weights must be converted to a wet tissue weight basis. If no conversion factor is reported with the BCF, multiply the dry weight by 0.1 for plankton and by 0.2 for species of fishes and invertebrates.

   e. If more than one acceptable BCF is available for a species, the geometric mean of values should be used, unless the BCFs are from different exposure durations, then the BCF for the longest exposure should be used.

4. If enough pertinent data exist, several residue values can be calculated by dividing maximum permissible tissue concentrations by appropriate BCFs:

   a. For each available maximum ADI derived from a feeding study or a long-term field study with wildlife, including birds and aquatic organisms, the appropriate BCF is based on the whole body of aquatic species which constitute or represent a major portion of the diet of tested wildlife species.

   b. For an FDA action level for fish or shellfish, the appropriate BCF is the highest geometric mean species BCF for the edible portion of a consumed species. The highest species BCF is used because FDA action levels are applied on a species-by-species basis.

5. For lipophilic materials, it may be possible to calculate additional residue values. Because the steady-state BCF for a lipophilic material seems to be proportional to percentage of lipids from one tissue to another and from one species to another (Hamelink et al. 1971, Lundsford and Blem 1982, Schnoor 1982), extrapolations can be made from tested tissues or species to untested tissues or species on the basis of percentage of lipids.

   a. For each BCF for which percentage of lipids is known for the same tissue for which the BCF was measured, normalize the BCF to a one percent lipid basis by dividing the BCF by percentage of lipids. This adjustment makes all the measured BCFs comparable regardless of species or tissue.

   b. Calculate the geometric mean normalized BCF.

   c. Calculate all possible residue values by dividing available maximum permissible tissue concentrations by the mean normalized BCF and by the percentage of lipids values appropriate to the maximum permissible tissue concentration.

      - For an FDA action level for fish oil, the appropriate percentage of lipids value is 100.

      - For an FDA action level for fish, the appropriate percentage of lipids value is 11 for freshwater criteria, based on the highest levels for important consumed species (Sidwell 1981).

- For a maximum ADI derived from a chronic feeding study or long-term field study with wildlife, the appropriate percentage of lipids is that of an aquatic species or group of aquatic species which constitute a major portion of the diet of the wildlife species.

6. The FRV is obtained by selecting the lowest of available residue values.

11. OTHER DATA

Pertinent information that could not be used in earlier sections may be available concerning adverse effects on aquatic organisms and their uses. The most important of these are data on cumulative and delayed toxicity, flavor impairment, reduction in survival, growth, or reproduction, or any other biologically important adverse effect. Especially important are data for species for which no other data are available.

12. CRITERION

1. A criterion consists of two concentrations: the Criterion Maximum Concentration and the Criterion Continuous Concentration.

2. The Criterion Maximum Concentration (CMC) is equal to one-half of the FAV.

3. The Criterion Continuous Concentration (CCC) is equal to the lower of the FCV, the Final Plant Value, and the FRV unless other data show a lower value should be used. If toxicity is related to a water quality characteristic, the CCC is obtained from the Final Chronic Equation, the Final Plant Value, and the FRV by selecting the value or concentration that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (see Section 11) show that a lower value should be used.

4. Round both the CCC and CMC to two significant figures.

5. The criterion is stated as:

The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses indicate that (except possibly where a locally important species is very sensitive) (1) aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of (2) does not exceed (3) µg/L more than once every three years on the average and if the one-hour average concentration does not exceed (4) µg/L more than once every three years on the average.

Where

(1) = insert freshwater or saltwater,

(2) = name of material,

(3) = insert the Criterion Continuous Concentration, and

(4) = insert the Criterion Maximum Concentration.

13. REFERENCES

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APPENDIX B:
SUMMARY OF USEPA METHODOLOGY FOR DETERMINING WATER QUALITY CRITERIA FOR THE PROTECTION OF HUMAN HEALTH

The following summary is a condensed version of the 1980 final US Environmental Protection Agency (USEPA) guidelines for calculating a water quality criteria to protect human health and is slanted towards the specific regulatory needs of the US Army. The guidelines are the most recent document outlining the required procedures and were published in the Federal Register (USEPA 1980). For greater detail on individual points consult that reference.

1. INTRODUCTION

The EPA's water quality criteria for the protection of human health are based on one or more of the following properties of a chemical pollutant:

a) Carcinogenicity, b) Toxicity, and c) Organoleptic (taste and odor) effects.

The meanings and practical uses of the criteria values are distinctly different depending on the properties on which they are based. Criteria based solely on organoleptic effects do not necessarily represent approximations of acceptable risk levels for human health. In all other cases the criteria values represent either estimations of the maximum allowable ambient water concentrations of a pollutant which would prevent adverse health effects or, for suspect and proven carcinogens, estimations of the increased cancer risk associated with incremental changes in the ambient water concentration of the substance. Social and economic costs and benefits are not considered in determining water quality criteria. In establishing water quality standards, the choice of the criterion to be used depends on the designated water use. In the case of a multiple-use water body, the criterion protecting the most sensitive use is applied.

2. DATA NEEDED FOR HUMAN HEALTH CRITERIA

Criteria documentation requires information on: (1) exposure levels, (2) pharmacokinetics, and (3) range of toxic effects of a given water pollutant.

2.1 EXPOSURE DATA

For an accurate assessment of total exposure to a chemical, consideration must be given to all possible exposure routes including ingestion of contaminated water and edible aquatic and nonaquatic organisms, as well as exposure through inhalation and dermal contact. For water quality criteria the most important exposure routes to be considered are ingestion of water and consumption of fish and shellfish.

Generally, exposure through inhalation, dermal contact, and non-aquatic diet is either unknown or so low as to be insignificant; however, when such data are available, they must be included in the criteria evaluation.

The EPA guidelines for developing water quality criteria are based on the following assumptions which are designed to be protective of a healthy adult male who is subject to average exposure conditions:

1. The exposed individual is a 70-kg male person (International Commission on Radiological Protection 1977).

2. The average daily consumption of freshwater and estuarine fish and shellfish products is equal to 6.5 grams.

3. The average daily ingestion of water is equal to 2 liters (Drinking Water and Health, National Research Council 1977).

Because fish and shellfish consumption is an important exposure factor, information on bioconcentration of the pollutant in edible portions of ingested species is necessary to calculate the overall exposure level. The bioconcentration factor (BCF) is equal to the quotient of the concentration of a substance in all or part of an organism divided by the concentration in ambient water to which the organism has been exposed. The BCF is a function of lipid solubility of the substance and relative amount of lipids in edible portions of fish or shellfish. To determine the weighted average BCF, three different procedures can be used depending upon lipid solubility and availability of bioconcentration data:

(1) For lipid soluble compounds, the average BCF is calculated from the weighted average percent lipids in ingested fish and shellfish in the average American diet. The latter factor has been estimated to be 3 percent (Stephan 1980, as cited in USEPA 1980)

Because steady-state BCFs for lipid soluble compounds are proportional to percent lipids, the BCF for the average American diet can be calculated as follows:

$$BCF_{avg} = BCF_{sp} \times \frac{3.0\%}{PL_{sp}},$$

where BCF_{sp} is the bioconcentration factor for an aquatic species and PL_{sp} is the percent lipids in the edible portions of that species.

(2) Where an appropriate bioconcentration factor is not available, the BCF can be estimated from the octanol/water partition coefficient (P) of a substance as follows:

$$\log BCF = (0.85 \log P) - 0.70$$

for aquatic organisms containing about 7.6 percent lipids (Veith et al. 1980, as cited in USEPA 1980). An adjustment for percent

lipids in the average diet (3 percent versus 7.6 percent) is made to derive the weighted average bioconcentration factor.

(3) For nonlipid-soluble compounds, the available BCFs for edible portions of consumed freshwater and estuarine fish and shellfish are weighted according to consumption factors to determine the weighted BCF representative of the average diet.

2.2 PHARMACOKINETIC DATA

Pharmacokinetic data, encompassing information on absorption, distribution, metabolism, and excretion, are needed for determining the biochemical fate of a substance in human and animal systems. Information on absorption and excretion in animals, together with a knowledge of ambient concentrations in water, food, and air, are useful in estimating body burdens in humans. Pharmacokinetic data are also essential for estimating equivalent oral doses based on data from inhalation or other routes of exposure.

2.3 BIOLOGICAL EFFECTS DATA

Effects data which are evaluated for water quality criteria include acute, subchronic, and chronic toxicity; synergistic and antagonistic effects; and genotoxicity, teratogenicity, and carcinogenicity. The data are derived primarily from animal studies, but clinical case histories and epidemiological studies may also provide useful information. According to the EPA (USEPA 1980), several factors inherent in human epidemiological studies often preclude their use in generating water quality criteria (see NAS 1977). However, epidemiological data can be useful in testing the validity of animal-to-man extrapolations.

From an assessment of all the available data, a biological endpoint, i.e., carcinogenicity, toxicity, or organoleptic effects is selected for criteria formulation.

3. HUMAN HEALTH CRITERIA FOR CARCINOGENIC SUBSTANCES

If sufficient data exist to conclude that a specific substance is a potential human carcinogen (carcinogenic in animal studies, with supportive genotoxicity data, and possibly also supportive epidemiological data) then the position of the EPA is that the water quality criterion for that substance (recommended ambient water concentration for maximum protection of human health) is zero. This is because the EPA believes that no method exists for establishing a threshold level for carcinogenic effects, and, consequently, there is no scientific basis for establishing a "safe" level. To better define the carcinogenic risk associated with a particular water pollutant, the EPA has developed a methodology for determining ambient water concentrations of the substance which would correspond to incremental lifetime cancer risks of 10^{-7} to

10^{-5} (one additional case of cancer in populations ranging from ten million to 100,000, respectively). These risk estimates, however, do not represent an EPA judgment as to an "acceptable" risk level.

3.1 METHODOLOGY FOR DETERMINING CARCINOGENICITY (NONTHRESHOLD) CRITERIA

The ambient water concentration of a substance corresponding to a specific carcinogenic risk can be calculated as follows:

$$C = \frac{70 \times PR}{q_1^* (2 + 0.0065BCF)}$$

where,
C = ambient water concentration;
PR = the probable risk (e.g., 10^{-5}; equivalent to one case in 100,000);
BCF = the bioconcentration factor; and
q_1^* = a coefficient (defined below) (USEPA 1980).

By rearranging the terms in this equation, it can be seen that the ambient water concentration is one of several factors which define the overall exposure level:

$$PR = q_1^* \times \frac{C (2 + 0.0065 BCF)}{70}$$

or

$$PR = q_1^* \times \frac{2C + (0.0065 BCF \times C)}{70},$$

where, 2C is the daily exposure resulting from drinking 2 liters of water per day and (0.0065 x BCF x C) is the average daily exposure resulting from the consumption of 6.5 mg of fish and shellfish per day. Because the exposure is calculated for a 70-kg man, it is normalized to a per kilogram basis by the factor of 1/70. In this particular case, exposure resulting from inhalation, dermal contact, and nonaquatic diet is considered to be negligible.

In simplified terms the equation can be rewritten

$$PR = q_1^* X,$$

where X is the total average daily exposure in mg/kg/day or

$$q_1^* = \frac{PR}{X},$$

showing that the coefficient q_1^* is the ratio of risk to dose; an indication of the carcinogenic potency of the compound.

The USEPA guidelines state that for the purpose of developing water quality criteria, the assumption is made that at low dose levels there

is a linear relationship between dose and risk (at high doses, however, there may be a rapid increase in risk with dose resulting in a sharply curved dose/response curve). At low doses then, the ratio of risk to dose does not change appreciably and q_1^* is a constant. At high doses the carcinogenic potency can be derived directly from experimental data, but for risk levels of 10^{-7} to 10^{-5}, which correspond to very low doses, the q_1^* value must be derived by extrapolation from epidemiological data or from high dose, short-term animal bioassays.

3.2 CARCINOGENIC POTENCY CALCULATED FROM HUMAN DATA

In human epidemiological studies, carcinogenic effect is expressed in terms of the relative risk [RR(X)] of a cohort of individuals at exposure X compared to the risk in the control group [PR(control)] (e.g., if the cancer risk in group A is five times greater than that of the control group, then RR(X) = 5). In such cases the "excess" relative cancer risk is expressed as RR(X) - 1, and the actual numeric, or proportional excess risk level [PR(X)] can be calculated:

$$PR(X) = [RR(X) - 1] \times PR(control).$$

Using the standard risk/dose equation:

$$PR(X) = b \times X$$

and substituting for PR(X):

$$[RR(X) - 1] \times PR(control) = b \times X$$

or

$$b = \frac{[RR(X) - 1] \times PR(control)}{X},$$

where b is equal to the carcinogenic potency or q_1^*.

3.3 CARCINOGENIC POTENCY CALCULATED FROM ANIMAL DATA

In the case of animal studies where different species, strains, and sexes may have been tested at different doses, routes of exposure, and exposure durations, any data sets used in calculating the health criteria must conform to certain standards:

1. The tumor incidence must be statistically significantly higher than the control for at least one test dose level and/or the tumor incidence rate must show a statistically significant trend with respect to dose level.

2. The data set giving the highest estimate of carcinogenic lifetime risk (q_1^*) should be selected unless the sample size is quite small and another data set with a similar dose-response relationship and larger sample size is available.

3. If two or more data sets are comparable in size and identical with respect to species, strain, sex, and tumor site, then the geometric mean of q_1^* from all data sets is used in the risk assessment.

4. If in the same study tumors occur at a significant frequency at more than one site, the cancer incidence is based on the number of animals having tumors at any one of those sites.

In order to make different data sets comparable, the EPA guidelines call for the following standardized procedures:

1. To establish equivalent doses between species, the exposures are normalized in terms of dose per day (m) per unit of body surface area. Because the surface area is proportional to the 2/3 power of the body weight (W), the daily exposure (X) can be expressed as:

$$X = \frac{m}{W^{2/3}}.$$

2. If the dose (s) is given as mg per kg of body weight:

$$S = \frac{m}{W},$$

then

$$m = s \times W$$

and the equivalent daily exposure (X) would be

$$X = \frac{(s \times w)}{W^{2/3}}$$

or

$$X = s \times W^{1/3}.$$

3. The dose must also be normalized to a lifetime average exposure. For an carcinogenic assay in which the average dose per day (in mg) is m, and the length of exposure is l_e, and the total length of the experiment is L_e, then the lifetime average exposure (X_m) is

$$X_m = \frac{l_e \times m}{L_e \times W^{2/3}}.$$

4. If the duration of the experiment (L_e) is less than the natural life span (L) of the test animal, the value of q_1^* is increased by a factor of $(L/L_e)^3$ to adjust for an age-specific increase in the cancer rate.

5. If the exposure is expressed as the dietary concentration of a substance (in ppm), then the dose per day (m) is

$$m = ppm \times F \times r,$$

where F is the weight of the food eaten per day in kg, and r is the absorption fraction (which is generally assumed to be equal to 1). The weight of the food eaten per day can be expressed as a function of body weight

$$F = fW,$$

where f is a species-specific, empirically derived coefficient which adjusts for differences in F due to differences in the caloric content of each species diet (f is equal to 0.028 for a 70-kg man; 0.05 for a 0.35-kg rat; and 0.13 for a 0.03-kg mouse).

Substituting (ppm x F) for m and fW for F, the daily exposure (dose/surface area/day or $m/W^{2/3}$) can be expressed as

$$X = \frac{ppm \times F}{W^{2/3}} = \frac{ppm \times f \times W}{W^{2/3}} = ppm \times f \times W^{1/3}.$$

6. When exposure is via inhalation, calculation can be considered for two cases: (1) the substance is a water soluble gas or aerosol, and is absorbed proportionally to to the amount of air breathed in and (2) the substance is not very water soluble and absorption, after equilibrium is reached between the air and the body compartments, will be proportional to the metabolic rate which is proportional to rate of oxygen consumption; which, in turn, is a function of total body surface area.

3.4 EXTRAPOLATION FROM HIGH TO LOW DOSES

Once experimental data have been standardized in terms of exposure levels, they are incorporated into a mathematical model which allows for calculation of excess risk levels and carcinogenic potency at low doses by extrapolation from high dose situations. There are a number of mathematical models which can be used for this procedure (see Krewski et al. 1983 for review). The EPA has selected a "linearized multi-stage" extrapolation model for use in deriving water quality criteria (USEPA 1980). This model is derived from a standard "general product" time-to-response (tumor) model (Krewski et al. 1983):

$$P(t;d) = 1 - \exp[-g(d)H(t)],$$

where P(t;d) is the probable response for dose d and

time t; g(d) is the polynomial function defining the effect of dose level, and H(t) the effect of time:

$$g(d) = \sum_{i=0}^{a} \alpha_i d^i,$$

$$H(t) = \sum_{i=1}^{b} \beta_i t^i,$$

(with α and $\beta \geq 0$, and $\sum \beta_i = 1$).

This time-to-response model can be converted to a quantal response model by incorporation of the time factor into each α as a multiplicative constant (Crump 1980):

$$P(d/t) = 1 - \exp\{ - \sum_{i=0}^{a} \alpha d^i \},$$

or as given in the EPA guidelines (USEPA 1980):

$$P(d) = 1 - \exp[-(q_o + q_1 d + q_2 d^2 + \ldots + q_k d^k)],$$

where P(d) is the lifetime risk (probability) of cancer at dose d.

For a given dose the excess cancer risk A(d) above the background rate P(o) is given by the equation:

$$A(d) = \frac{P(d) - P(o)}{1 - P(o)},$$

where

$$A(d) = 1 - \exp[-q_1 d + q_2 d^2 + \ldots + q_k d^k)],$$

Point estimates of the coefficients $q_1 \ldots q_k$ and consequently the extra risk function A(d) at any given dose are calculated by using the statistical method of maximum likelihood. Whenever q_1 is not equal to 0, at low doses the extra risk function A(d) has approximately the form:

$$A(d) = q_1 \times d.$$

Consequently, $q_1 \times d$ represents a 95 percent upper confidence limit on the excess risk, and R/q_1 represents a 95 percent lower confidence limit on the dose producing an excess risk of R. Thus A(d) and R will be a function of the maximum possible value of q_1 which can be determined from the 95 percent upper confidence limits on q_1. This is accomplished by using the computer program GLOBAL 79 developed by Crump and Watson (1979). In this procedure q_1^*, the 95 percent upper confidence limit, is calculated by increasing q_1 to a value which, when incorporated into the log-likelihood function, results in a maximum value satisfying the equation:

$$2(L_o - L_1) = 2.70554,$$

where L_o is the maximum value of the log-likelihood function.

Whenever the multistage model does not fit the data sufficiently, data at the highest dose are deleted and the model is refitted to the data. To determine whether the fit is acceptable, the chi-square statistic is used:

$$\chi^2 = \sum_{i=1}^{h} \frac{(X_i - N_i P_i)^2}{N_i P_i \times (1 - P_i)},$$

where N_i is the number of animals in the ith dose group, X_i is the number of animals in the ith dose group with a tumor response, P_i is the probability of a response in the ith dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups.

The fit is determined to be unacceptable whenever chi-square (X^2) is larger than the cumulative 99 percent point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of nonzero multistage coefficients.

4. HEALTH CRITERIA FOR NONCARCINOGENIC TOXIC SUBSTANCES

Water quality criteria that are based on noncarcinogenic human health effects can be derived from several sources of data. In all cases it is assumed that the magnitude of a toxic effect decreases as the exposure level decreases until a threshold point is reached at, and below which, the toxic effect will not occur regardless of the length of the exposure period. Water quality criteria (C) establish the concentration of a substance in ambient water which, when considered in relation to other sources of exposure [i.e., average daily consumption of nonaquatic organisms (DT) and daily inhalation (IN)], place the Acceptable Daily Intake (ADI) of the substance at a level below the toxicity threshold, thereby preventing adverse health effects:

$$C = \frac{ADI - (DT + IN)}{[2L + (0.0065\ kg \times BCF)]}$$

where 2L is the amount of water ingested per day, 0.0065 kg is the amount of fish and shellfish consumed per day, and BCF is the weighted average bioconcentration factor.

In terms of scientific validity, an accurate estimate of the ADI is the major factor in deriving a satisfactory water quality criteria.

The threshold exposure level, and thus the ADI, can be derived from either or both animal and human toxicity data.

4.1 NONCARCINOGENIC HEALTH CRITERIA BASED ON ANIMAL TOXICITY DATA (ORAL)

For criteria derivation, toxicity is defined as any adverse effects which result in functional impairment and/or pathological lesions which may affect the performance of the whole organism, or which reduce an organism's ability to respond to an additional challenge (USEPA 1980).

A bioassay yielding information as to the highest chronic (90 days or more) exposure tolerated by the test animal without adverse effects (No-Observed-Adverse-Effect-Level or NOAEL) is equivalent to the toxicity threshold and can be used directly for criteria derivation. In addition to the NOAEL, other data points which can be obtained from toxicity testing are

(1) NOEL = No-Observed-Effect-Level,
(2) LOEL = Lowest-Observed-Effect-Level,
(3) LOAEL = Lowest-Observed-Adverse-Effect-Level,
(4) FEL = Frank-Effect-Level.

According to the EPA guidelines, only certain of these data points can be used for criteria derivation:

1. A single FEL value, without information on the other response levels, should not be used for criteria derivation because there is no way of knowing how far above the threshold it occurs.

2. A single NOEL value is also unsuitable because there is no way of determining how far below the threshold it occurs. If only multiple NOELs are available, the highest value should be used.

3. If a LOEL value alone is available, a judgement must be made as to whether the value actually corresponds to a NOAEL or an LOAEL.

4. If an LOAEL value is used for criteria derivation, it must be adjusted by a factor of 1 to 10 to make it approximately equivalent to the NOAEL and thus the toxicity threshold.

5. If for reasonably closely spaced doses only a NOEL and a LOAEL value of equal quality are available, the NOEL is used for criteria derivation.

The most reliable estimate of the toxicity threshold would be one obtained from a bioassay in which an NOEL, NOAEL, LOAEL, and clearly defined FEL were observed in relatively closely spaced doses.

Regardless of which of the above data points is used to estimate the toxicity threshold, a judgement must be made as to whether the experimental data are of satisfactory quality and quantity to allow for a valid extrapolation for human exposure situations. Depending on whether the data are considered to be adequate or inadequate, the

toxicity threshold is adjusted by a "safety factor" or "uncertainty factor" (NAS 1977). The "uncertainty factor" may range from 10 to 1000 according to the following general guidelines:

1. Uncertainty factor 10. Valid experimental results from studies on prolonged ingestion by man, with no indication of carcinogenicity.

2. Uncertainty factor 100. Data on chronic exposures in humans not available. Valid results of long-term feeding studies on experimental animals, or in the absence of human studies, valid animal studies on one or more species. No indication of carcinogenicity.

3. Uncertainty factor 1000. No long-term or acute exposure data for humans. Scanty results on experimental animals with no indication of carcinogenicity.

Uncertainty factors which fall between the categories described above should be selected on the basis of a logarithmic scale (e.g., 33 being halfway between 10 and 100).

The phrase "no indication of carcinogenicity" means that carcinogenicity data from animal experimental studies or human epidemiology are not available. Data from short-term carcinogenicity screening tests may be reported, but they are not used in criteria derivation or for ruling out the uncertainty factor approach.

4.2 CRITERIA BASED ON INHALATION EXPOSURES

In the absence of oral toxicity data, water quality criteria for a substance can be derived from threshold limit values (TLVs) established by the American Conference of Governmental and Industrial Hygienists (ACGIH), the Occupational Safety and Health Administration (OSHA), or the National Institute for Occupational Safety and Health (NIOSH), or from laboratory studies evaluating the inhalation toxicity of the substance in experimental animals. TLVs represent 8-hr time-weighted averages of concentrations in air designed to protect workers from various adverse health effects during a normal working career. To the extent that TLVs are based on sound toxicological evaluations and have been protective in the work situation, they provide helpful information for deriving water quality criteria. However, each TLV must be examined to decide if the data it is based on can be used for calculating a water quality criteria (using the uncertainty factor approach). Also the history of each TLV should be examined to assess the extent to which it has resulted in worker safety. With each TLV, the types of effects against which it is designed to protect are examined in terms of its relevance to exposure from water. It must be shown that the chemical is not a localized irritant and there is no significant effect at the portal of entry, regardless of the exposure route.

The most important factor in using inhalation data is in determining equivalent dose/response relationships for oral exposures. Estimates of equivalent doses can be based upon (1) available pharmacokinetic data for oral and inhalation routes, (2) measurements of absorption efficiency from ingested or inhaled chemicals, or (3) comparative excretion data when associated metabolic pathways are equivalent to those following oral ingestion or inhalation. The use of pharmacokinetic models is the preferred method for converting from inhalation to equivalent oral doses.

In the absence of pharmacokinetic data, TLVs and absorption efficiency measurements can be used to calculate an ADI value by means of the Stokinger and Woodward (1958) model:

$$ADI = TLV \times BR \times DE \times d \times A_A/(A_O \times SF),$$

where,

BR = daily air intake (assume 10 m^3),
DE = duration of exposure in hours per day,
d = 5 days/7 days,
A_A = efficiency of absorption from air,
A_O = efficiency of absorption from oral exposure, and
SF = safety factor.

For deriving an ADI from animal inhalation toxicity data, the equation is:

$$ADI = C_A \times DE \times d \times AA \times BR \times 70\ kg/(BWA \times AO \times SF),$$

where,

C_A = concentration in air (mg/m^3),
DE = duration of exposure (hr/day),
d = number of days exposed/number of days observed,
AA = efficiency of absorption from air,
BR = volume of air breathed (m^3/day),
70 kg = standard human body weight,
BWA = body weight of experimental animals (kg),
AO = efficiency of absorption from oral exposure, and
SF = safety factor.

The safety factors used in the above equations are intended to account for species variability. Consequently, the mg/surface area/day conversion factor is not used in this methodology.

5. ORGANOLEPTIC CRITERIA

Organoleptic criteria define concentrations of substances which impart undesirable taste and/or odor to water. Organoleptic criteria are based on aesthetic qualities alone and not on toxicological data, and therefore have no direct relationship to potential adverse human health effects. However, sufficiently intense organoleptic effects may,

under some circumstances, result in depressed fluid intake which, in turn, might aggravate a variety of functional diseases (i.e., kidney and circulatory diseases).

For comparison purposes, both organoleptic criteria and human health effects criteria can be derived for a given water pollutant; however, it should be explicitly stated in the criteria document that the organoleptic criteria have no demonstrated relationship to potential adverse human health effects.

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